SIZE INHERITANCE AND GEOMETRIC GROWTH PROCESSES IN THE TOMATO FRUIT

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INTRODUCTION

THE literature on size inheritance contains but few references to what seems to be an important clue to its study, namely, a frequent tendency for F_1 and F_2 hybrids to approach the geometric mean of the parent sizes (GROTH 1914, 1915). It is almost universally assumed and taught that the ideal hybrid will "blend" in size to the arithmetic mean. The reasons for the comparative neglect of geometric relationships seem to be mainly that the studies of size inheritance hitherto made have been with few exceptions confined to crosses between parents so similar in size that arithmetic and geometric means are not easily distinguished. Such confusion is but natural, since size fluctuates widely under environmental influences, and is often confusingly increased over its true value by heterosis.

The classic data are in fact mainly drawn from crosses where the parent races or species differed by not more than 2, 3 or 4 to 1. Approximately such a ratio characterizes the parental differences in the bulk of the published crosses involving linear measurements or proportions, surface areas, volumes or weights of the body as a whole or of some part or organ. Witness the many studies on rodents and fowls among animals and the even more numerous similar observations on the inheritance of size of flowers, seeds, fruits, leaves, stems or roots of both cultivated and wild plants.

Much greater size differences than these are obtainable in crossable parents in selected cases. For instance artificial selection has produced an extremely large size range in fruits of apples, peppers and tomatoes, in tubers of potatoes, and a striking contrast in body size between bantam and Asiatic breeds of fowls, etc. Hybrids between such extremes generally show not blending but "dominance of small size," or inheritance by some geometric rule.

The tomato fruit provides especially favorable material for size study, since there are enormous differences between the small one gram fruits of "Red Currant" (*Lycopersicon pimpinellifolium*) and the comparatively gigantic 100 to 400 gram fruits of some commercial varieties of *L. esculentum*. During the last twelve years we have accumulated a vast amount of size data, and from them evolved a theory of size inheritance (MACARTHUR 1935, BUTLER 1937), here applied in the hope that it will clarify

TABLE 1

Mean fruit weight in grams of the different tomato crosses.

CROSS NUMBER	LARGER PARENT	SMALLER PARENT	NO. OF F_2 PLANTS	LARGE P_1	SMALL P_1	F_1	F_2	GEOMETRIC MEAN	ARITHMETIC MEAN
Parents Differing Greatly in Size									
449	Yellow Pear	×Red Currant	84	12.4	1.1	4.2	4.2	3.7	6.7
3413	Large Pear	×Red Currant	210	54.1	1.1	7.4	6.4	7.4	27.6
25A	Grape Cluster	×Red Currant	75	55.8	1.1	7.9	8.0	7.6	28.5
3313	902 selection	×Red Currant	914	56.2	1.1	7.2	7.3	7.6	28.6
34T	Putman's Forked	×Red Currant	124	57.0	1.1	7.1	7.5	7.7	29.0
3316	745 selection	×Red Currant	650	57.5	1.1	7.4	7.8	7.7	29.3
452	Honor Bright	×Red Currant	143	150.0	1.1	9.4	6.9	12.3	75.5
441	Golden Queen	×Red Currant	136	152.4	1.1	10.1	10.2	12.3	76.7
3210	Tangerine	×Red Currant	720	173.6	1.1	8.3	9.5	13.2	87.3
414	Albino	×Red Currant	219	312.0	1.1	12.6	12.3	17.5	156.5
3415	Tangerine	×*r y f H* selection	140	173.6	5.0	38.1	50.0	29.4	89.3
729	*p o r y* selection	×Burbank Pres.	58	36.0	5.1	14.0	15.8	13.5	20.6
901	*l c u h* selection	×Burbank Pres.	238	55.0	5.1	21.0	22.3	16.8	30.0
721	Devon Surprise	×Burbank Pres.	66	58.0	5.1	23.0	22.9	17.2	32.5
403	Dwarf Aristocrat	×Yellow Pear	222	112.6	12.4	35.5	41.9	37.4	62.5
445	McMullen Pink	×Yellow Pear	286	148.8	12.4	44.0	45.2	43.0	80.6
451	Honor Bright	×Yellow Pear	136	150.0	12.4	47.5	46.1	43.3	81.2
Parents Differing Slightly in Size									
3409	*r y f H* selection	×Red Currant	86	5.0	1.1	3.3	3.4	2.5	3.0
405	Peach	×Yellow Pear	495	42.6	12.4	23.1	26.2	23.0	27.5
410	Honor Bright	×White Apple	183	150.0	39.1	49.9	57.2	76.5	94.5
401	Dwarf Aristocrat	×Peach	163	112.4	42.6	67.1	73.3	69.5	77.5
450	Honor Bright	×Peach	390	150.0	42.6	68.1	61.6	80.0	96.3
434	Dwarf Aristocrat	×Grape Cluster	90	112.4	55.8	73.3	81.1	79.0	84.1
3209	Tangerine	×902 selection	534	173.6	56.2	112.3	86.3	99.0	114.9
3201	Tangerine	×R. N. H.	350	173.7	68.0	91.2	81.1	109.0	120.8
409	Albino	×Dw. Aristocrat	216	312.0	112.4	137.9	160.4	188.0	212.2
438	Albino	×Honor Bright	80	312.0	150.0	160.0	151.3	217.0	231.0
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some aspects of the problem and also suggest other avenues of research. As a good deal of this material is embodied in the thesis to be published later by the junior author, this paper will be confined to a statement of the theory with a modicum of supporting data. The observations will be presented first and will treat the subject from four different angles.

1. THE INHERITANCE OF FRUIT WEIGHT

There is a large amount of variation between the weights of fruits even on the same plant, but, as will be shown in a later paper, each variety or inbred line has its own average inherited weight. By crossing lines of different fruit weights we can see how the various contrasting fruit sizes are inherited. The average fruit weight was determined by weighing ten fruits from each plant, a certain amount of selection being exercised to avoid using obviously atypical fruits. These abnormal fruits were of two types, undersized ones due to faulty pollination or lop-sided growth, and those that were oversized owing to an increase in the locule number.

The crosses presented (table 1) are divided into two categories, first the cases where the two parents differ greatly in fruit size, and second, those in which the parents differ only slightly. It will be noticed that the resulting hybrids cover the whole range of possible behaviors, illustrating at the one extreme the geometric mean and at the other extreme typical blending. Closer scrutiny of this table will show that the cases that conform most closely to the arithmetic mean of the two parents are crosses in which the parents did not differ greatly in fruit size (small by small, medium by medium, or large by large). It will also be noted that only one F_1 weight is given; it was found that even when the parental size contrast was greatest the reciprocal crosses never differed significantly in F_1 fruit weight.

The main purpose of the table is to bring out the close agreement in both the interspecific and intraspecific crosses between the observed F_1 and F_2 means and the calculated geometric means, and the poor agreement with the arithmetic means. The greater the parental contrasts, the larger become the discrepancies between arithmetic and geometric values and the more the F_1 and F_2 values favor the geometric mean.

That a similar geometric relationship applies equally well in the case of backcrosses is shown in table 2.

Analyzed from this point of view numerous crosses and "grading up" backcrosses in other species show a similar behavior. This is illustrated in SAUNDER'S and MACOUN'S breeding project with the wild Siberian crab; by large commercial varieties of apples (CHIPMAN 1933); in peppers (DALE 1929); and in F_1 hybrids of bantam fowls by large Asiatic breeds (JULL and QUINN 1931).

In these varied materials the approach of the F_1, F_2 and backcross sizes to the geometric means may be taken to be the general rule, the seeming approach to the arithmetic mean being really limited to those special cases in which parental differences are comparatively small. Since the latter happen to be most numerous in the genetic literature they have come to be considered typical, possibly obscuring thereby an essential feature of size inheritance.

TABLE 2

Mean fruit weights in grams of two tomato crosses and their respective backcrosses.

	P_1	B.C.	F_1, F_2	B.C.	P_1
Red Current×Tangerine	1.12±.005	3.14±.09	9.03±.13	32.2±.78	173.6±3.6
Geometric mean		3.19	13.8	39.5	
Arithmetic mean		5.08	87.3	91.3	
Red Current×902 selection	1.12±.005	2.5±.15	7.41±.10	19.8±.95	56.2±.05
Geometric mean		2.9	7.9	20.4	
Arithmetic mean		4.27	28.6	31.8	

2. THE F_2 DISTRIBUTION OF FRUIT WEIGHTS

A prime essential for the study of problems of inheritance of size and other important features of organization is the merging of the physiological, developmental and genetic points of view. The problem thus becomes primarily one of determining the physiology of gene effects during the development of such quantitative characters.

In the recent literature there has been a tendency to attack the problem from the standpoint of gene action and to subject the older view of blending inheritance to considerable criticism. KAPTEYN (1916) long ago pointed out, with numerous appropriate biological examples, that causes depending on size produce proportional or positively skew distributions, instead of normal frequency curves. RASMUSSON'S (1933) theory of genic interaction assumes that the total effect of the genotype is not determined by the direct simple addition of the effects of quantity genes, but that they interact with one another in such a way that the cumulative effect of all the genes is less than the sum of their individual effects; the net result is inevitably a negatively skew curve. POWERS (1936) in his work on barley actually obtained data directly opposing this view and concluded that the cumulative effect of a number of genes was greater than the sum of their individual effects. Recently SINNOTT (1937) has shown that his F_2 frequency distributions in summer squashes are positively skewed and has interpreted this to indicate that the effects of the size genes are geometrically cumulative.

The form of the F_2 distribution is thus often used as an index of the type of inheritance and the mode of operation of the size genes; for these purposes data are given from two F_2 populations, each consisting of more than a thousand plants. In both cases the curves (fig. 1) are positively skew, and on the basis of such large numbers this skewness is significant. Since in cases of blending inheritance a normal curve is always attained

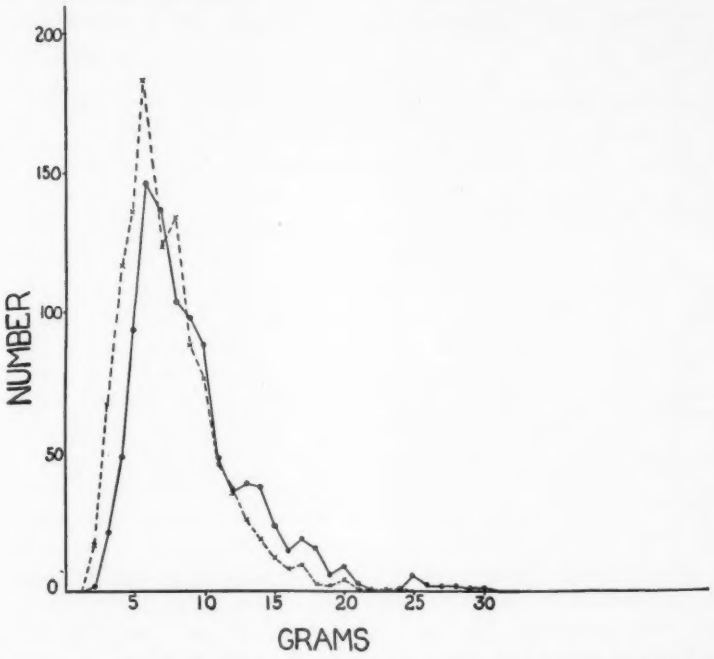

FIGURE 1. Positively skew distributions of mature fruit weights (grams) in two F_2 tomato populations:

Cross	Number of plants	Arithmetic means	Geometric means	Median	$g_1=\frac{k_3}{k_2^{3/2}}$
3313 F_2 ---x---	1107	7.41±.10	6.73	6.24	+1.105
3210 F_2 —·—	994	9.03±.09	8.24	7.49	+1.596

unless interaction takes place, some explanation must be given for the skewness. Obviously RASMUSSON's theory does not apply in this case. But if the factors react with one another on a geometric or percentage basis, each adding a definite proportion to the already existing capital, then such addition to a small capital will make very little difference, while the same percentage increases based on a large capital will result in large increases.

The result of such a scheme will be that the weights crowd together at the lower end of the scale and spread out at the upper end. This is just what happens in many of the crosses that we have studied. From the means and statistical constants of the data given in the legend of figure 1 it will be seen that the geometric means of the distributions are much closer to the median than are the arithmetic means. This would indicate that the distribution is almost normal around the geometric mean, so that if the data were plotted logarithmically the curve would be nearly symmetrical.

TABLE 3

Mean fruit weights in grams of tall and dwarf F_2 segregates, and their absolute and percentage differences.

Tall parent	Dwarf parent	Mean F_2 fruit weights		Differences	
		Tall	Dwarf	Absolute (grams)	Proportional (percent)
Red Currant	×902 selection	6.9± .10	8.4± .30	1.4± .31	*20.5*
Tangerine	×902 selection	81.8±1.4	101.6±2.0	19.8±2.5	*23.0*
Devon Surprise	×B. Preserving	24.0±1.0	18.1±2.5	5.9±2.7	*26.2*
Yellow Peach	×Dwarf Aristocrat	76.8±1.5	61.2±1.9	15.6±2.4	*21.1*
740 selection	×"Primula"	24.8±1.4	18.0±1.3	6.8±1.9	*30.0*
97M selection	×3213 selection	54.0±1.4	38.0±2.3	16.0±2.7	*32.0*

3. THE LINKAGE OF QUALITATIVE AND QUANTITATIVE FACTORS

The results of LINDSTROM (1928) and our own extensive data (mainly unpublished) show that there are a number of definite genetic linkages between the known qualitative factors and fruit size genes; for the present purpose part of the first chromosome linkages are chosen (table 3) to illustrate some novel features of gene relationship. These are all crosses of a dwarf parent with a tall one; in some cases the fruits of the dwarf F_2 segregates are larger than those of the tall ones, while in other cases, where expected, the converse relationship is true. Therefore this linkage of fruit size with the dwarf gene must be interpreted as truly genetic and not physiological. The important point now concerns a comparison of the values in the last two columns of table 3. It will be noted that, whereas the absolute difference in grams (next to the last column) between the means of the two segregate classes varies a great deal, the percent increases (in the last column) are relatively constant. Since this percentage is found by putting the difference between the class means over the F_2 mean it would appear probable that the action of the true fruit size gene or genes linked in this first chromosome is to cause an increase in growth which is proportional to the basic fruit size. This illustrates clearly the

difference in such a situation between simple additive growth and proportional or geometric growth. The geometric type of increase prevails in this case, and our results show further that the same type of relationship is exhibited with at least eleven other genes.

Not only the true size genes, but also those influencing size through shape, or general physiological effects display the same action. In our more recent studies (MACARTHUR 1934 a, 1935) we have observed that at least two factors, fasciated fruit (*f*) and lutescent foliage (*l*), show a physiological association with size. The fasciation factor always tends to make the fruits of the fasciated segregates larger than those of the respective smooth-fruited ones, regardless of whether the fasciated parent is the larger or the smaller fruited. Lutescent, on the other hand, tends to make the fruits smaller than those on the green plants in the same F_2. It is interesting to note that though the differences of the F_2 class means of the fasciated and smooth in the one case and the lutescent and green in the other are erratic when considered in terms of grams, they are rational and subject to analysis when considered on a percentage basis. The rough fasciated fruits are 60 to 80 percent larger than the regular smooth ones, while the fruits of the lutescent plants are 15 to 20 percent smaller than those of the corresponding green plants, carrying similar residual genes.

It is interesting to anticipate here what is taken up more fully in section four and the discussion, namely, the physiological mechanism involved. The evidence to date regarding the action of the fasciated gene indicates that it exerts its effect in the early primordial stage by proliferating more locules; and indeed it would seem logical to infer from observations of the number of fasciated flowers that contain two, three, or even four separate or partly fused ovaries, that the mechanism is more properly characterized as ovary proliferation rather than locule proliferation. No matter which of these mechanisms is operating the net result of imposing this system on the basic fruit size is the same; that is, the effect of this increase in locule number on final fruit size is proportional to the already determined locule size and final cell size. Lutescent on the other hand seems to retard the later developmental processes so that its effect is also proportional to the capital involved.

4. THE GROWTH OF THE TOMATO FRUIT AS RELATED TO CELL NUMBER AND CELL SIZE

Since 1925 it has been our established practice (without at first realizing its significance) to classify tomato plants segregating for fruit shapes (oval, pear, fasciated, etc.) and in a general way for fruit size by an inspection of the ovary primordium at anthesis. This naturally directed attention to growth phenomena during the early period, and in the summer of 1934

the junior author made a histological study of cell number and cell size during this period and of the influence of these factors on the subsequent development of fruit size. Since the majority of our conclusions, though derived in a different manner, are essentially the same as Miss HOUGHTALING'S (1935) the reader is referred to her paper for a detailed discussion of the influence of cell number and cell size on the mature fruit size.

Our conclusions may be summarized as follows:—

1. The period prior to anthesis is characterized by cell division, and any differences in ovary size at anthesis are associated entirely with cell number and not with cell size.
2. The post-anthesis period is characterized chiefly by cell expansion, cell division being a minor factor which just suffices to maintain the epidermis, the cells of which do not expand.
3. The differential cell expansion takes place early in the post-anthesis period and the maximum cell expansion varies greatly in the several varieties.

Since in arriving at these conclusions we made use of the probable number of cells present at each stage, some of the figures of which are included in table 4, it is advisable to give the method employed in calculating them. The variety Yellow Cherry has an ovary diameter of 1.1 mm and a cell wall thickness of .1 mm, the diameter of the cells at this stage is .01 mm. From these data the following computations are made, assuming the fruit to be a sphere:—

External volume $1.1^3 \times .5236$	$= .6969$	cu. mm
Internal volume $(1.1-.2)^3 \times .5236$	$= .3817$	cu. mm
Volume of ovary wall	$= .3152$	cu. mm
Volume of cell $.01^3$	$= .000001$	cu. mm
Number of cells $.3152/.000001$	$= 315{,}000$	cells.

By the above method of calculation the cells in the central septa are omitted but this does not introduce any serious error, and in our estimation is preferable to any method that treats the ovary as a solid body.

Table 4 shows that the tomato species and varieties can be divided into three more or less well defined groups as regards cell numbers, with *L. pimpinellifolium* representing the first group, yellow cherry the second, and the *esculentum* varieties the third. The two F_1 hybrids between Red Currant and *esculentum* types as well as Burbank Preserving, known to be a selection from such a cross, fall into the second or intermediate group. Our more recent data show that all F_1 hybrids of *L. pimpinellifolium* X *L. esculentum* have cell numbers between 300,000 and 400,000 and fall into this class, even when the *esculentum* variety is Beefheart which has a relatively enormous 400 gram fruit.

In general the larger the fruit the greater the number of cells in its ovary; there are however exceptions to this rule which are explained by reference to the fifth column where the diameters of the mature cells are given. Therefore cell number and cell expansion together account for most of the final fruit size, as may be tested by computing the cell mass (from the diameters) and multiplying by the cell number.

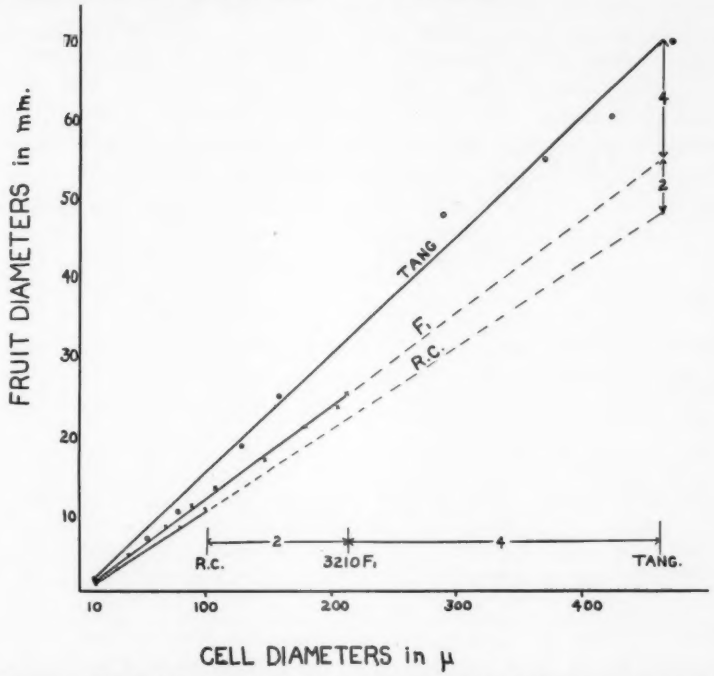

FIGURE 2. Graphs indicating that cell expansion and cell size both operate as geometric processes. A differential increase in cell numbers is seen in the divergent slopes of the three lines and a differential cell expansion in unequal extensions of these lines (see text).

As cell expansion also plays an important part in the determination of fruit size something should be said about its mode of inheritance. Table 4 shows that the mature cell diameters are also divisible into three classes and that these classes bear a geometric relationship to one another.

The effect of these two factors, cell number and cell size, on the final fruit size is brought out clearly in figure 2, where cell diameter is plotted against fruit diameter. The relationship between cell and fruit diameter is evidently a straight line, the slope of the line in the case of the two parents being very different and the F_1 being intermediate. The differences

in slope indicate that for any given cell size the Tangerine has a much larger fruit than does the Red Currant, because of the greater number of cells present in the Tangerine variety. The amount of divergence between the three lines is therefore the effect of initial cell number; the greater the cell expansion the larger the absolute differences between the three lines, but the relative differences remain the same. By observing the end points on each line the effect of cell expansion on fruit size can be studied. If we hold cell number constant, by plotting the final cell sizes all on a line with the same slope, such as the extrapolated Red Currant and F_1 lines,

TABLE 4

The number of cells in the pericarp wall of tomato fruits at flowering time and the cell diameters in mature fruits. The cell size at anthesis in all varieties is taken as .01 mm.

VARIETY	FRUIT WEIGHT (GRAMS)	OVARY DIAMETER (MM)	THICKNESS OF WALL (MM)	NUMBER OF CELLS IN THOUSANDS	MATURE CELL DIAMETERS (MICRA)
Red Currant	1.1± .005	.85	.086	164	110
3313 F_1 (902×R.C.)	7.2± .06	1.08	.10	303	200
3210 F_1 (Tang×R.C.)	8.3± .07	1.09	.10	309	220
Yellow Cherry	4.2± .11	1.1	.10	315	120
Burbank Preserving	5.2± .09	1.2	.09	401	140
Yellow Peach	42.6±2.0	1.5	.08	507	400
Devon Surprise	58.0±2.2	1.4	.10	531	400
Banalbufor	33.1±1.0	1.5	.10	617	350
902 selection	56.2± .05	1.6	.08	640	400
Stirling Castle	98 ±5.4	1.7	.08	659	360
Tangerine	173 ±3.6	1.8	.08	745	550
John Baer	69.6±3.4	1.8	.09	829	470

we find that the fruit diameters read from these points show geometric progression. Hence we are dealing with two geometric factors, which together would account for the observed relationship between parents and F_1 shown in table 1, and the effect of cell expansion superimposed on cell number is to accentuate the geometric relationship. A similarity may be seen in the time of operation of these two factors, differential cell division taking place in the early part of the pre-anthesis period, and differential cell expansion taking place in the early part (first nine days) of the post-anthesis period.

From the P_1 and F_1 data there at first appeared to be a correlation between cell number and cell size. But in the F_2 population the apparent association between cell number and cell size proved to be a pseudo-correlation; for when the weight is held constant by partial correlation methods

we may safely conclude that cell number and cell expansion are really two separate genetic entities.

Letting w = mature weight, s = cell size, and n = cell number, the correlations are as follows:

$$r_{ws} = +.674 \pm .038$$
$$r_{sn} = +.411 \pm .059 \qquad r_{sn.w} = +.0009 \pm .70$$
$$r_{wn} = +.523 \pm .051$$

Thus the study of cell number and size, and of the growth of the tomato fruit through all but the earliest primordial stages of its development, has led to some definite results which may be associated with the genetic findings. Parents of different fruit size, and their reciprocal crosses in F_1, F_2 and backcross generations come early to differ in cell numbers in their growth. This early difference in cell number is followed by a subsequent brief period of differential cell expansion. The growth in the long final period is approximately equal both in rate and duration in all varieties and hybrids.

The data presented under headings 1 to 4 draw attention to four lines of approach to the problem of fruit size inheritance, each leading to the same conclusion. Collectively they form a substantial support to the following hypothesis.

A WORKING HYPOTHESIS OF FRUIT SIZE INHERITANCE

A view has already been proposed (MacArthur 1935, Butler 1937) that appears tenable for the tomato data and adequate to explain the principal results described. The basic size factors are considered as rate genes, one group of which governs the rate of division (or the duration of active mitosis) in the formative stages from the earliest establishment of the fruit primordium to anthesis. Earlier or later in this limited time-effective period a large-fruited variety or species undergoes a few more cell divisions than a smaller-fruited one. The differences of cell numbers found in fruits of typical varieties (table 4) are such as would be caused by a difference of one or two divisions out of at the most 17–19 divisions. Each extra division at this stage doubles the initial capital. Ovary proliferation and formation of additional locules may both increase this capital. On the basis of such differences in cell number at anthesis, the size increases occurring later by expansion of each of the cells is therefore proportional to the cell number. Increase of capitals at equal rates over equal periods of time by the compound interest rule results in comparatively vast absolute differences in final fruit sizes. There is, however, the further evidence suggesting that other factors governing cell expansion itself may be also geometric in their action (fig. 2). Since both basic processes, con-

trolling cell number and cell size, are probably geometric in nature, the mature fruit sizes would be such as were observed (tables 1 and 2).

From the genetic aspect, the allelic size factors from parents of different size would control the rate and number of divisions in the corresponding formative period of the F_1 hybrid. Thus a blending of mitotic rates results in the "dominance of small size" (wild type) factors in the fruit of the hybrid, that is, in a geometric mean of ultimate size as far as cell number is involved; a partial dominance of the slower rate would reduce the F_1 size even below the geometric mean (table 1).

A small parent with cells from x mitotic divisions crossed with a large parent with $x+2$ divisions would produce an F_1 with fruit size characterized by $x+1$ divisions. This latter number is also the average of the F_2 generation, but with factor segregation increasing the variability as in the usual theory. To illustrate with an actual case, the cross of the 164,000-celled Red Currant with the 745,000-celled Tangerine was observed to produce an F_1 hybrid bearing fruits of 309,000 cells. In the two backcrosses the expected less-than-intermediate number of cells are evidently formed, as shown by the ovary sizes and the geometric means.

Similar considerations are obviously applicable to cell expansion (data in last column of table 4). In any case a large amount of variation in fruit sizes would be expected for at least two reasons, quite apart from the known modifying effects of environment and of genes controlling shape, locule number, etc (YEAGER 1937): (1) Doubtless mitoses do not continue synchronously in all cells of the anlage, at least after chemo- and histodifferentiation begin. Accordingly cell numbers in varieties and hybrids are not expected to conform strictly to any 1:2:4 series. (2) Cell division and cell expansion appear to be largely independent processes; if so, the possible combinations of cell numbers and sizes would be numerous and varied enough to give a wide range of fruit sizes. An attempt is being made to synthesize and select some of these combinations, for example fruits containing the Red Currant cell number and the Tangerine cell size, etc. Each such selection should theoretically be obtainable with or without fasciation, with high or low locule number, and in varying shapes. Collectively these selections would be expected to duplicate most of the fruit types occurring in the known cultivated varieties and wild species.

DISCUSSION

The familiar view of size inheritance in its simplest form assumes that size genes 1) are numerous, 2) lack dominance, producing a blend as regards size in the F_1 hybrid, 3) are equal, and 4) simply additive in their effect, producing symmetrical F_2 distributions, and that 5) their segrega-

tion and assortment explain the increased variance of the F_2 population and the different means and variance of F_3 selections.

Objection is raised only to attributes 2), 3) and 4). The assumed lack of dominance is patently inconsistent with the observed behavior of the bulk of qualitative genes and with the dominance theory of heterosis, which also deals with the same or similar quantitative factors. The cases actually analyzed have often revealed some major and other accessory or modifying size genes. The F_2 distributions are frequently asymmetrical.

In the tomato where the analysis of fruit size has perhaps been carried farther than in other species, genetic studies have from the first shown clearly that the fruit size is gene-transmitted, size as a character being unusually complex and multifactorial. Many typical recessive mutant factors affecting size have been recognized and located in chromosomes (MACARTHUR 1934b); some of these exert their important size effects by modifying fruit shape or locule number or both (YEAGER 1937); for example, the genes for fasciation (*f*) and those raising the locule number increase fruit size, probably by increasing the basic cell number, while at least two genes which elongate the fruit (ovate, *o*, and "plum") tend to decrease its size. Other recessive genes with marked and distinguishing specific qualitative effects also have a general or physiological influence on size (MACARTHUR 1934a, 1935; CASTLE 1936); lutescent (*l*), a chlorophyll deficiency, slows down both plant and fruit growth and decreases the ultimate size of the fruit; tangerine (*t*) and possibly peach (*p*), on the other hand, appear to enlarge the fruit perhaps by acting on the cell expansion mechanism. Linkage experiments have shown the existence of other factors, for example, in chromosomes I, II and III, which presumably act as "size genes" *per se* for they produce their size effects when fruit shape, locule number, and all known qualitative factors are held constant.

These instances show that the several identified factors controlling size obviously do not necessarily or always lack dominance. Many are known to exhibit a fairly typical dominant-recessive relationship. It is relevant to mention also that the dominance theory of hybrid vigor implies an F_2 asymmetry, but not of the kind observed, since the skewness is negative if large size is dominant (ASHBY 1937c, pp. 432–33).

Some of the factors isolated are certainly unequal, for they have been shown to affect size by varying amounts. Several of the genes determining size or number of cells are apparently distinct in nature and mode of influence, inasmuch as they affect different processes and act at different times in ontogeny. The histological analysis of fruit development, though still far from complete, already directs attention not to one process only, but to many (rate and duration and localization of cell division, rate of

advancing differentiation, time and amount of cell expansion, etc). It is certainly clear that some of the chief size factors influence ovary proliferation (*f*), or locule number, others localization of differential growth (*f*, *o*, plum); and still others probably determine fruit cell expansion (*t*, *p*) or fruit cell division rate or general plant growth (*l*). These factors being dissimilar in nature and unequal in effect, no linear relation would be expected or possible between the number of factors and the size of the fruits. What they have in common is that most of them act comparatively early in fruit growth, and probably all of them act, whether directly or indirectly, on processes of a geometric nature.

Since the F_2 distributions are strongly and positively skew the interaction of the various size factors can hardly be either arithmetically cumulative in total effect or according to a law of diminishing returns. Both the F_1 positions and the F_2 distributions are features associated with geometric growth, and show the likelihood that consideration of geometric processes will prove indispensable for an understanding of size inheritance. Since cell number and size are of widespread importance in many processes involving embryological organization and physiological functions, it is possible that quantitative characters other than size may also have a geometric basis.

It deserves some notice that conclusions in many ways parallel have been reached recently by several investigators working with quite different materials and objectives. In mutant races of Drosophila, an eye size directly proportional to that characteristic of the imago has been fixed and is already detectable in the anlage by the time the first 11.6 percent of the total development period is completed (MEDVEDEV 1935). The breed differences in body size of rabbits are evident when only five or six cleavage divisions have occurred (CASTLE and GREGORY 1929, GREGORY and CASTLE 1931); and in chickens such cell number differences are discernible in the embryos before hatching (BLUNN and GREGORY 1935). The latest work on the physiology of heterosis (ASHBY 1937a, b, c; LUCKWILL 1937; but contrast EAST 1936) has again referred the greater size of F_1 plants displaying a marked hybrid vigor to an initial advantage in size of the embryo primordium. Such cases from both animals and plants suggest that some common and consistent explanation may be found to account for the genetically determined size differences characteristic of parts or of whole organisms.

These observations concentrate attention on the present need for researches 1) to discover just how early and by what factors the differences in amount of capital, that is, the number and size of cells comprising the primordium of the organ anlage or embryo, are determined; 2) to demonstrate more precisely by linkage studies the existence and nature of factors

more or less specifically controlling cell number and cell size, and obtain such factors in their various combinations, and 3) to unify the theory underlying heterosis and quantitative inheritance in general.

SUMMARY AND CONCLUSIONS

The tomato provides especially favorable material for the study of fruit size inheritance from the combined genetic and developmental aspects. Many qualitative and quantitative factors have been identified and mapped whose specific or general size effects have been localized as to time, site and general mode of action.

That fruit size fundamentally involves geometric processes is indicated by four different lines of approach:

1) The average F_1, F_2 and backcross fruit sizes approach more closely to the geometric mean of the parent varieties or species crossed, than to their arithmetic means, as is usually supposed.

2) Linked size genes, as well as fruit shape and other genes affecting size, appear to operate on a percentage basis.

3) The F_2 distributions are positively skew.

4) Histological analysis of developing fruits shows that the basic phenomena involved are those controlling cell number and cell size, both of which appear to act during limited time-effective periods (p. 260), and in a geometrical manner.

The characteristic variety or species fruit sizes are anticipated in the size of the ovary primordium, which in turn is determined by differences in cell number (brought about by different rates of cell division in the preanthesis period), and by varying amounts of cell expansion (fixed in the first days after anthesis). On the basis of the unequal cell numbers and sizes established in the early anlage the later observed proportionate or percentage increases produce relatively enormous absolute differences in mature fruit size.

A theory proposed to account for the histological observations and the genetic results is that rate genes control in the main two basic geometric processes, some determining the number of cell divisions, others the amount of cell expansion. Together the factors govern differential growth in size of the primordia, creating unequal amounts of initial capital. It is such genes acting geometrically the heterozygous combinations of which produce the F_1 fruit sizes noted and the assortment of which explains the means and skewness of the F_2 distributions.

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THE RELATION OF ALBINISM TO BODY SIZE IN MICE

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IN PREVIOUS studies made in coöperation with former colleagues at the BUSSEY INSTITUTION of HARVARD UNIVERSITY, it was found that certain recessive mutant genes, when homozygous, increase the body size of mice, whereas other mutant genes decrease the body size.

In particular the brown (or chocolate) gene was found to increase adult body weight by 3 or 4 percent, and body length by about 1.5 percent. That the brown mutation increases body size was independently discovered and first reported by FELDMAN (1935), who made observations on the relative body weight of black and of brown individuals in three different races of mice in which the two alternative colors were occurring together in the same litter. He gives averages for a group of from 35 to 50 mice of each sex and color, weighed at monthly intervals between the ages of one month and six months. At each of the six weighings in both sexes, the brown mice were heavier than the blacks by from 1.6 to 5.6 percent. Combining the percent differences for both sexes, the series runs thus:

Age in months	1	2	3	4	5	6
Browns heavier, in percent	2.8	2.2	2.0	3.1	2.4	3.5

From this it would seem that the brown gene, in FELDMAN's observations as in our own, when homozygous makes mice heavier by about 3 percent than when it is heterozygous, and further that this influence is of about the same strength at all ages. The growth period studied by FELDMAN covers that from an average weight of about 9 grams at one month of age to a weight of about 23 grams at six months of age in males of race H. In race J the corresponding weights are 10 and 32, and in the third race (IHJ) they are 9.8 and 26.6.

From the fact that the percentage difference is substantially the same at 1, 4, and 6 months of age in FELDMAN's mice, it seems probable that it was already effective at birth or even earlier (as genetic size differences are in rabbits).

The dilution gene also was found by us to increase body weight and body length, though to a less extent than the brown gene, but tail length was in several crosses increased more strongly by dilution than by brown, indicating a special localized action of the dilution gene.

The combined action of the two genes, brown and dilution, was about equal to the sum of their effects when acting separately.

An opposite effect, decrease of body size was found to occur, when the gene for short ear or the gene for pink eye was present in homozygous state. The gene for short ear was thought to reduce body weight by about 5 percent, the gene for pink eye by less than one percent, though the evidence was not altogether clear.

In the case of the agouti gene, no evidence was found that it either increases or decreases body size.

In continuance of this line of research, an experiment has recently been made to ascertain what effect, if any, the albino mutation *c* has on body size. The conclusion reached is that it has no effect, since individuals homozygous for albinism do not differ in average size from their colored litter mates which are heterozygous for albinism. The evidence on which this conclusion is based will now be presented.

In the fall of 1936, in the Veterinary Science Laboratory of the UNIVERSITY OF CALIFORNIA, a cross was made between albino mice of the formula *AA bb cc* and dilute brown colored mice of the formula *aa bb CC*. The albino mice were kindly supplied by Dr. E. C. MACDOWELL, the dilute brown mice were obtained from the Supply Department of the ROSCOE B. JACKSON MEMORIAL LABORATORY. Both races had been long inbred and so would be theoretically of complete genetic uniformity. In making the cross albino females were mated with dilute brown males. The F_1 young, like their albino mothers, were animals of remarkable size, vigor, and fecundity. In color they were cinnamon and their genetic formula obviously would be *Aa bb Cc*. That is, they were heterozygous for agouti and for albinism, but like both parents were homozygous for the brown gene.

F_1 females were now backcrossed with males of a triple recessive albino race kindly supplied by Dr. L. C. STRONG, his long inbred race A, which is of the formula *aa bb cc*. The resulting backcross mice fall into four genetic classes expected to be numerically equal one to another, *Aa bb Cc* (cinnamon), *aa bb Cc* (brown), *Aa bb cc* (albinos potentially cinnamon), and *aa bb cc* (albinos potentially brown). The last two classes are indistinguishable in appearance and so there are really only three phenotypes, cinnamon, brown, and albino, and their expected proportions are 1:1:2. In a backcross population of 1252 mice raised to an age of six months, the actual numbers are 334 cinnamon, 308 brown, and 610 albino, a sufficiently close approximation to the expected 1:1:2 ratio.

A comparison of individuals of the three phenotypes should show whether genes *A*,*a* and *C*,*c*, in their alternative forms exercise any appreciable influence on body size. If the gene *A* exercises any influence on size different from that of its allele, *a*, then the cinnamon mice (*Aa*) should differ significantly in size from the browns (*aa*). And if gene *C* exercises

on size an influence different from that of its allele, *c*, then the average size of the colored mice (cinnamons and browns, which agree in being *Cc* in formula) should be different from that of the albinos, which are all *cc* in formula.

Animals of the backcross population were weaned at an age of three or four weeks. The sexes were caged separately, about 12 or 15 animals to a cage, and kept constantly supplied with Purina Dog Chow and water. They were weighed individually at monthly intervals from about four months of age, and the maximum weight recorded for each animal was regarded for statistical purposes as its adult weight. In the case of females, which were of course not allowed to breed, the final weight observation made was usually the maximum, or at any rate there was little decline up to six months of age from a maximum previously attained. In the case of males the maximum was often attained as early as four months of age, subsequent to which weight might be lost from fighting but this did not seem to affect either body length or tail length, if the tail remained uninjured. In case the tail was severely injured by fighting, its length was not included in the calculation of average tail length. This accounts in part for the smaller number of animals tabulated as to tail length. But there also occurred a certain number of animals in both sexes which had stubby tails obviously abbreviated at birth by an overzealous mother in the process of cleaning the new born young, or else congenitally shorter and stubbier than normal as to tail form. These also were omitted in tabulating the data on tail length.

The tail length in these backcross mice was measured from the point to which the body fur covers the tail (disregarding the longest contour hairs) to the tail tip (projecting hairs however being here disregarded). The tail measurement was made independently of the body measurement and the difficulty in determining the point on the *morphological* tail to which the body fur extended will account in part (but only in part) for the greater variability of the tail measurement, as compared with that of body length. Actually tail length varies more in relation to body length, than body weight does. This is indicated by the lesser magnitude of the correlation coefficient between tail and body when compared with the correlation coefficient between weight and body. Weighing was done with a Toledo scale, which proved both expeditious and accurate to within 0.2 gram.

When the animals were six months old they were chloroformed and measurements were taken of the body length and tail length of each animal after SUMNER's method, keeping the body slightly stretched under tension of 20 gram weights attached to teeth and tail respectively.

Table 1 contains a summary, for each sex separately, of the observa-

tions on body weight, body length, and tail length. Males are in all three respects larger than females and so are summarized separately, but no phenotype differs significantly from either of the other phenotypes of the same sex in weight, body length, or tail length.

Brown males average a trifle larger bodied by all three criteria than cinnamon males, but the difference is less than twice the probable error

TABLE 1

Comparative body size in a backcross population, of mice of the three phenotypes, cinnamon, brown and albino, as indicated by body weight, body length and tail length.

MALES	NO.	AVERAGE WEIGHT	NO.	AVERAGE BODY LENGTH	NO.	AVERAGE TAIL LENGTH
Cinnamon	173	39.45±.16	172	102.66±.10	165	93.75
Brown	153	39.91±.14	153	102.92±.10	146	93.82
Cin. and br. combined	326	39.67±.09	325	102.78±.07	311	93.77
Albino	310	39.54±.09	310	102.58±.07	304	93.82
Total	**636**	**39.61±.05**	**635**	**102.68±.03** σ=1.90±.03	**615**	**93.79±.07** σ=2.91±.05
FEMALES						
Cinnamon	161	30.78	161	98.38	161	90.89
Brown	155	31.34	155	98.10	154	90.68
Cin. and br. combined	316	31.09	316	98.22	315	90.78
Albino	300	31.47	300	98.75	295	91.02
Total	**616**	**31.27**	**616**	**98.33**	**610**	**90.90**

and so not significant. And in the case of females, this relation is reversed at least as regards body length and tail length, for cinnamon females exceed their brown sisters slightly in these measurements. We may conclude therefore that the *Aa* phenotype does not differ in body size from the *aa* phenotype, which conclusion agrees with that reached in experiments previously reported.

We come now to the prime objective of this experiment, to discover whether albinism has a tendency either to increase or to decrease body size. For this purpose we may compare the average body size of colored individuals with that of their albino litter mates. The combined cinnamon and brown classes constitute the colored individuals, the body size of which is to be compared with that of the albinos. The 326 colored males of table 1 have an average body weight of 39.67 grams; the 310 albino males average 39.54 grams. The difference between these averages is .13 gram, which scarcely exceeds the probable error, .12 gram, and so is not significant.

In body length, the colored males are just .10 mm longer bodied than the albinos, a difference which just equals the probable error. In tail length the difference between colored and albino males is also insignificant, being only .05 gram, actually less than the probable error. Albinism accordingly is without detectable influence on the body size of males.

A similar conclusion is reached in the case of females from a comparison of the body size of colored and of albino females. The average body weight of 316 colored females is 31.09 grams. For 300 albino females, it is .38 gram greater but this is less than the difference in weight between the two colored classes, cinnamon and brown, which on grounds already discussed was not considered significant. Also the relation between the colored and the albino females as regards body weight is the reverse of that observed among the males, since colored males were heavier than albino males, but colored females weigh less than albino females. Probable errors were not calculated for the female population, but if they are substantially the same as for the corresponding groups of males, the difference in weight between colored and albino groups would not have statistical significance.

The albino females, as regards body length and tail length, as well as body weight, are slightly larger bodied than their colored sisters, but this relation is doubtless a consequence of random sampling and not indicative of genetic differences, as is shown by the following considerations. As the data were accumulated, they were from time to time summarized. Three such partial summaries were made, about 200 individuals being included in each. In two of these summaries the average weight of the brown females was greater than that of the cinnamons, but in the third summary the cinnamons were heavier than the browns. Also in two of the partial summaries colored females are heavier than albinos, but in the third summary albinos are heavier than colored individuals.

That it is through general rather than local growth processes that genes commonly influence body size is indicated by the positive correlations which exist between body weight, body length, and tail length. An individual which is large by one of these criteria is also large by the other criteria, and an individual which is small by one criterion is also small by the others. This is true even within inbred races and populations derived by crossing such inbred races, as in the present experiment. Here genetic uniformity is nearly complete and such variability as exists must be referred almost wholly to accidents of development. Organic correlations are regularly less within inbred populations where genetic influences are uniform than in other populations in which genetic influences are variable. For example, in the backcross population described by CASTLE, GATES and REED (1936), where several genes affecting body size were segregating, the correlation between body weight and body length was found to be

$.65 \pm .01$ in the case of females, and $.66 \pm .01$ in the case of males. But in the present experiment in which variation is uninfluenced by genes affecting body size, the corresponding correlation for males is only $.55 \pm .01$.

Tail length shows a greater degree of independent variability than either weight or body length. The correlation between body length and tail length was found to be only $.26 \pm .02$ in the available male population of 613 individuals, in which were included only those with uninjured tails. In a previous publication the gene mutation dilution was found to exert a direct influence on tail length, in addition to the indirect influence which in common with the brown mutation it exerts through its action on general growth. In the present experiment homozygous dilution does not occur in the backcross population, so this complication is avoided.

SUMMARY

An experimental test was made of the influence of the albino mutation on the body size of mice. Albino females of an inbred race (*AAbbcc*) were crossed with dilute brown males (*aabbCC*). The F_1 mice, cinnamon in color, were *AabbCc*. F_1 females were backcrossed to triple recessive inbred males *aabbcc*. Mice of three phenotypes were produced, cinnamon (*AabbCc*), brown (*aabbCc*), and albinos (either *Aabbcc* or *aabbcc*). These three phenotypes in a backcross population of 1252 animals occurred in the expected ratio, 1:1:2. The animals were grown under uniform conditions to an age of six months, then killed and measured as to body length and tail length, having been previously weighed at monthly intervals. The body size of each individual was judged by three criteria, maximum weight at or prior to six months of age, body length and tail length.

No significant difference was found between body size, as estimated by any one of these criteria, among the three phenotypes. In particular the colored classes were neither larger nor smaller bodied than the albinos. The conclusion is reached that albinism (and incidentally also the non-agouti mutation) is without influence on body size. Among the 635 male individuals the correlation between weight and body length was found to be $.55 \pm .01$. Among 613 available males the correlation between body length and tail length was found to be $.26 \pm .02$.

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NEW MUTANTS IN SCIARA AND THEIR GENETIC BEHAVIOR

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THE present paper deals with six new mutant characters in Sciara found in this laboratory. In addition, two mutants are recorded which appear to be identical with or allelic to two of these characters. Of the six, one is an autosomal dominant in *Sciara coprophila* Lintner, and one a sex-linked recessive in *Sciara ocellaris* Comstock. The remaining four, including one autosomal dominant, two sex-linked dominants, and one sex-linked recessive, are in *Sciara reynoldsi* Metz (METZ, in press). Selective segregation of chromosomes has previously been established in three species of Sciara (METZ 1926, 1928, 1929). The present paper establishes selective segregation in two other species; namely, *S. ocellaris* and *S. reynoldsi*.

The dominant characters were found by the second author among the descendants of flies which had been exposed to radium. The radiation was done at the Howard A. Kelly Hospital in Baltimore through the kindness of Dr. FRED WEST, to whom we are greatly indebted. Whether the mutations were produced as a result of the radiation cannot be stated definitely,

TABLE 1

Linkage data for the character "Stop" in S. coprophila.

TEST	CASES	TOTAL FLIES	CLASSIFICATION OF FLIES				PERCENTAGE OF CROSSING OVER
			D	*F*	*DF*	+	
D×*F*	16	1177	297	279	301	300	Not linked
			S	*C*	*SC*	+	
S×*C*	9	405	109	73	80	143	Not linked
			S	*F*	*SF*	+	
S×*F*	18	688	338	349	1	0	0.15
			S	*Dl*	*SDl*	+	
S×*Dl*	32	1160	587	570	1	2	0.26
			S	*B*	*SB*	+	
S×*B*	22	852	445	404	0	3	0.35
			S	*D*	*SD*	+	
S×*D*	12	393	152	190	21	30	12.98
			S	*tr*	*Str*	+	
S×*tr*	24	1357	563	414	183	197	28.00

since Sciara has proved to be very resistant to radiation. Since the characters are dominants, it seems probable that some of them, at least, were produced at the time of radiation. Otherwise, they should have appeared previously in the stock cultures.

The sex-linked recessive in *S. reynoldsi* was found by the first author, and the genetical tests, analyses, and descriptions presented here (with the exception of the sex-linked recessive in *S. ocellaris*, which was found and analyzed by Mrs. E. GAY LAWRENCE) are by the first author.

I. *The mutant character Stop in S. coprophila*

a) *Origin.* Stop is an autosomal dominant which was found on February 20, 1936, in a female, the mother of which had been treated with 8 gm. hrs. of radium.

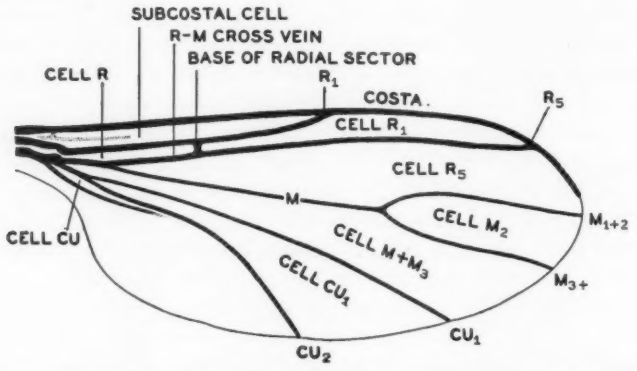

FIGURE 1. Diagram of normal Sciara wing. Terminology after Johannsen. R1, first branch of radius; Rs, posterior branch of radius; M, media; M1+2, anterior branch of media; M3+, posterior branch of media; Cu1, anterior branch of cubitus; Cu2, posterior branch of cubitus.

b) *Description.* In the Stop wing (see fig. 1 for diagram of normal Sciara wing) the posterior branch of the radius vein and the anterior and posterior branches of the media vein end before they reach the wing margin. Stop is a clear-cut and constant character, which can be recognized easily in crosses that involve other wing characters. Stop flies exhibit excellent viability as shown by the fact that heterozygous Stop females crossed to wild type males give progenies which closely approximate a 1:1 ratio. Seven pair matings of this type selected at random gave 132 wild type to 118 Stop flies.

c) *Genetic behavior of males.* The transmission of Stop through males follows the typical Sciara pattern, that is, males transmit only the genes derived from their mothers. From crosses of wild type virgin females to

Stop males which received Stop from their mothers, the offspring are all Stop. On the other hand, the offspring from crosses of wild type virgin females to Stop males which received Stop from their fathers, are all wild type.

d) *Linkage.* Genetic tests, corroborated by cytological observations, as shown below, indicate that Stop involves a translocation between chromosomes II and IV.

Crosses of virgin wild type females to Stop males (which received Stop from their mother) revealed that Stop was not sex-linked. If it were sex-linked, the F_1 males from such a cross would all be wild type, since the paternal X is eliminated from the soma cells in the male at about the eighth cleavage division (DUBOIS 1933). The F_1 males from such a cross, however, are all Stop, indicating that the Stop character is an autosomal dominant. Three autosomal linkage groups have been identified in *S. coprophila* (SMITH-STOCKING 1936): chromosome II, with the characters *truncate* and *Dash;* chromosome III, with the *Curly* character; and chromosome IV, with *Blister, Delta,* and *Fused.*

(1) *Tests with Chromosome III:* Stop×Curly (*S*×*C*). Tests summarized in table 1 show that Stop is not in Chromosome III.

(2) *Tests with Chromosome IV:* Stop×Fused (*S*×*F*). The data, given in table 1, indicate that Stop is "in" chromosome IV and closely linked to Fused.

Stop×Delta (*S*×*Dl*), and Stop×Blister (*S*×*B*). Similarly, Stop was found to be linked to Delta and Blister. From table 1 we find the crossover value between Stop and Delta to be 0.26 percent, while that between Stop and Blister is 0.35 percent. Delta and Blister are probably alleles. SMITH-STOCKING (1936) reports 6.9 percent crossing over between Delta and Fused and 7.0 percent crossing over between Blister and Fused.

(3) *Tests with Chromosome II:* Stop×Dash (*S*×*D*). Crosses between Stop and Dash revealed that Stop is linked also to a character in chromosome II. Tests between Stop and Dash gave a crossover value of 12.98 percent between Stop and Dash. The question arose as to whether Dash was really in a linkage group distinct from Blister, Delta, and Fused. SMITH-STOCKING'S report (1936) gives clear-cut evidence that neither Blister nor Delta is linked to Dash; however, no direct tests between Fused and Dash are recorded. In order to clarify this point, tests were made between Dash and Fused. Classifying the offspring from these crosses was somewhat complicated since Fused obliterates one of the characteristic effects of the Dash gene, that is, the "Dash" vein situated between the anterior and posterior branches of the cubitus vein. However, Fused does not obliterate the brownish marking along the posterior branch of the radius which is also a characteristic effect of Dash. The tests be-

tween Dash and Fused indicate definitely that Dash and Fused are located in different chromosomes.

Stop×truncate (*S*×*tr*). Tests between Stop and truncate gave further proof that Stop is linked to characters in chromosome II as well as to those in chromosome IV. Such tests gave a crossover value of 28 percent between Stop and truncate.

e) *Conclusion.* In view of the genetic evidence presented above, Stop is believed to be due to (or accompanied by) a translocation between chromosomes II and IV. Cytological examination of the salivary gland chromosomes of heterozygous Stop flies shows that there is a translocation between two of the four pairs of synapsed somatic chromosomes. This translocation makes it possible to identify cytologically the four linkage groups in *S. coprophila;* the translocation distinguishes chromosomes II and IV, and the X chromosome is distinguishable from chromosome III by its unpaired condition in the male soma (that is, in salivary gland cells).

The Stop translocation apparently affects crossing over in both chromosomes, II and IV. In chromosome IV SMITH-STOCKING (1936) found 6.9 percent crossing over between Delta and Fused and 7.0 percent crossing over between Blister and Fused. From table 1 we find the crossover value between Stop and Fused to be 0.15 percent, between Stop and Delta 0.26 percent, and between Stop and Blister 0.35 percent. It appears, therefore, that the Stop translocation cuts down crossing over in chromosome IV. On the other hand, Stop apparently increases crossing over in chromosome II, since the crossover value between Stop and Dash is 12.98 percent and that between Stop and truncate 28 percent; while the crossover value between truncate and Dash reported by SMITH-STOCKING (1936) is only 2.8 percent.

II. The mutant character "yellow" in S. ocellaris

a) *Origin.* A yellow male was found in a normal wild type culture of *Sciara ocellaris* on February 11, 1935 by Mrs. E. GAY LAWRENCE.

b) *Description.* Yellow is a body color which extends to most parts of the fly except the eyes and bristles. It is clear-cut and constant and can be readily distinguished from wild type.

c) *Genetic behavior.* Yellow behaves as a sex-linked recessive (first analyzed by Mrs. E. G. LAWRENCE). Yellow females from the monogenic strain give either male or female families (a few "exceptional" males and females are found). Thus, if a yellow female-producing female is mated to wild type males, all of her daughters should be phenotypically wild type. On the other hand, if a yellow male-producing female is mated to wild type males, all of her sons should be yellow, since the paternal sex chromosome is normally eliminated from the male soma at an early cleav-

age stage (DuBois 1933). However, it may be mentioned here that there are many cases of irregular sex-chromosome elimination in this yellow strain (unpublished data of Mrs. E. Gay Lawrence and of Crouse). For instance, a number of males have been found which retained the paternal sex chromosome in their soma instead of the maternal sex chromosome.[1] Normally Sciara males transmit only those characters which are maternal in origin. An attempt has been made to determine whether these irregular males which retain the paternal sex chromosome in the soma line transmit this chromosome, but all such males tested have proved to be sterile.

III. Mutant characters in S. reynoldsi

1. Puff

a) *Origin.* Puff is an autosomal dominant which appeared in a digenic strain of *S. reynoldsi.* It was found on January 4, 1936 in a male, the mother of which had been treated with 5 grm. hrs. of radium.

b) *Description and genetic behavior.* Puff is very similar to the character "Blister" in *S. coprophila.* It appears as a blister or vesicle at the juncture of the posterior and anterior branches of the media vein. The Puff character is variable; it ranges all the way from a large blister to a slight swelling, and in some cases overlaps normal. Fifteen pair matings of Puff females × wild type males, selected at random, gave the following offspring: 294 wild type females, 204 Puff females, 283 wild type males, 165 Puff males, that is, approximately 63 offspring per female. On the other hand, fifteen pair matings selected at random of wild type females by wild type males gave 515 females and 903 males in the F_1, that is, approximately 95 offspring per female.

Homozygous Puff females have never been found. Puff females derived from the cross, Puff female × Puff male (Puff from mother), have all proved to be heterozygous. Apparently, therefore, Puff is lethal in homozygous condition. The transmission of Puff through the male line agrees with that of other characters in Sciara. Puff males which receive the character from their mother transmit it to all their offspring, whereas Puff males which receive Puff from their father do not transmit it at all.

2. Vesiculated

a) *Origin.* A Vesiculated female was found on January 6, 1936 in a digenic culture of *S. reynoldsi.* The parents of this Vesiculated fly had been exposed to 3 grm. hrs. of radium.

[1] Such irregular elimination does not appear to be associated with the yellow locus itself, since the paternal sex chromosome retained in some cases has carried yellow and in other cases its normal allele.

b) *Description and genetic behavior.* In appearance, Vesiculated is identical with Puff; it is likewise variable, ranging all the way from a large blister to a normal juncture of the posterior and anterior branches of the media vein. Like Puff, Vesiculated is lethal in homozygous condition. However, it is much less viable when heterozygous. From ten pair matings selected at random of Vesiculated females×wild type males, there were 123 wild type females, 72 Vesiculated females, 64 wild type males, and 35 Vesiculated males in the F_1, that is, approximately 29 offspring per female. To determine whether Puff and Vesiculated are alleles is practically impossible, since the two characters are so similar. However, Puff females were mated to Vesiculated males (the reciprocal cross was made also) and all of the F_1 females backcrossed to wild type. Without exception, each mating gave two types of offspring: wild type and Puff (or Vesiculated?). That is, in no case were Puff and Vesiculated obtained in the same fly. This fact, together with the fact that neither Puff nor Vesiculated has been found in homozygous condition, suggests that the two characters are allelic or perhaps identical. The considerable difference in viability makes it probable that they are not identical.

3. Jagged and Jagged-2

a) *Origin.* A Jagged female appeared in a digenic culture of *S. reynoldsi* on January 6, 1936. Its parents had been treated with 5 grm. hrs. of radium.

b) *Description.* The Jagged character appears as nicks or incisions along the wing margin between the posterior branch of the cubitus vein and the anterior branch of the media vein. The position and number (usually one or two) of these marginal nicks are somewhat variable, and in some instances the Jagged character is detectable only by missing marginal hairs. Jagged showed poor viability from the time of its origin, and the stock was finally lost.

c) *Genetic behavior.* The first Jagged female found was mated to wild type males. The offspring from this cross fell into three groups: wild type females, Jagged females, and wild type males. During the eighteen months that Jagged was kept in the laboratory, the offspring from Jagged females mated to wild type males always fell into these three groups. No Jagged males ever appeared. This fact indicates that Jagged is a sex-linked dominant which is lethal in homozygous condition, and also lethal in the male where one sex chromosome is present in the soma.

d) *Jagged-2.* On the same day that Jagged appeared, Jagged-2 was found in an F_1 female of the same digenic stock of *S. reynoldsi.* The parents of this J-2 fly had been treated with 5.4 grm. hrs. of radium. The J-2

character proved to be identical with Jagged in appearance, viability, and genetic behavior.

4. Ruffled

a) *Origin.* A Ruffled male was found in January, 1936, in a digenic strain of *S. reynoldsi*. The parents of this male had been treated with 2.9 grm. hrs. of radium.

b) *Description.* In the female heterozygote the wings appear stiff and are characterized by a wave approximately midway of their length. The degree of waviness varies, and in many cases the female heterozygotes are not distinguishable from wild type. On the other hand, the female homozygotes never overlap normal. The wings of these flies are wavy, extremely wrinkled, and have something of the appearance of the wings of newly-hatched imagos. In other words, the wing size, shape, and venation are altered in the female homozygotes. The wing of the Ruffled male seems to occupy a position intermediate between these heterozygotic and homozygotic extremes. The wings are only slightly wrinkled, and the venation can be seen. However, the wave in the wing is distinct, and Ruffled males can always be distinguished from wild type.

c) *Genetic behavior.* Ruffled shows the same type of inheritance as other sex-linked dominants in Sciara.

5. Yellow

a) *Origin.* A yellow Ruffled male was found in a mass culture of Ruffled stock on October 23, 1936 by the first author.

b) *Description.* Yellow in *reynoldsi*, like yellow in *ocellaris*, is a body color which extends to most parts of the fly except the eyes and bristles.

c) *Genetic behavior.* The original yellow Ruffled male was mated to wild type females. The F_1 generation consisted of 60 Ruffled females and 33 wild type males, showing that yellow is a recessive. These Ruffled F_1 females backcrossed to wild type males gave, without exception, four classes of offspring: wild type males, yellow Ruffled males, wild type females, and Ruffled females. Yellow thus behaved as a sex-linked recessive. When these F_2 Ruffled females (carrying yellow) were mated to yellow Ruffled males, yellow Ruffled females, Ruffled females, yellow Ruffled males, and wild type males were obtained. During the twelve months that yellow has been kept in the laboratory, it has always appeared with Ruffled. Evidently little, if any, crossing over occurs between the two loci. Due to the decreased viability of homozygous Ruffled, the stock carrying yellow has to be kept outcrossed. Apparently the yellow gene does not decrease viability, since yellow Ruffled flies are as viable as Ruffled flies.

The fact that *S. ocellaris* and *S. reynoldsi* can be hybridized (METZ and LAWRENCE in press) has made it possible to cross *ocellaris* yellow and *reynoldsi* yellow and to demonstrate that the two genes are alleles.

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The authors are indebted to Dr. C. W. METZ for suggestive criticism and to Mrs. ELIZABETH GAY LAWRENCE for making some of the genetic tests.

SUMMARY

Six new mutants have been found in Sciara, among descendants of radium-treated flies. Stop, an autosomal dominant in *S. coprophila*, involves a translocation between chromosomes II and IV. Yellow is a sex-linked recessive in *S. ocellaris*. Four mutations have been found in *S. reynoldsi:* Puff and Vesiculated, autosomal dominants which are lethal when homozygous and may be allelic or perhaps identical; Jagged, a sex-linked dominant, lethal when homozygous; Ruffled, a sex-linked dominant; and yellow, a sex-linked recessive closely linked to Ruffled.

The yellow mutations of *S. reynoldsi* and *S. ocellaris* are alleles.

The genetic behavior of these characters establishes selective segregation in *S. ocellaris* and *S. reynoldsi*.

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SALIVARY CHROMOSOME STUDIES OF LETHALS IN DROSOPHILA MELANOGASTER

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IT IS very difficult to define a lethal except in the wide genetical sense of being a factor which brings about the death of the organism. Supposedly every genetic lethal has its physiological or morphological counterpart which prevents the organism from living beyond a certain stage, but little is known about these lethals themselves—whether they are genic or chromosomal changes, are closely allied to mutations, are additions to or losses from the non-lethal constitution. The evidence in Drosophila indicates that the types of changes which are genetically detectable form a graded series; cases are known from one extreme, where the change results in decreased viability, to the other extreme, where the change is both lethal to the organism as a whole and to the individual cell, and all intermediate types have been encountered. Thus lethals can be classified according to the degree of their effect. Even more complex is any attempt to correlate genetic lethals with cytological changes since cases are known of lethals appearing in salivary gland chromosomes as deficiencies (SLIZYNSKA 1938), of genetically tested lethals having no effect on the known banding (SLIZYNSKA 1938), and of visible cytological deficiencies not having any lethal effect at all (DEMEREC and HOOVER 1936). It is clear then that the study of lethals is from many points of view an interesting one. The approach to these problems chosen here is that of parallel genetical and cytological studies which are possible in *Drosophila melanogaster*. This organism is particularly suitable for such a study because the frequency of occurrence of both spontaneous and induced lethals is relatively high.

PROBLEM AND MATERIAL

In the study of lethals interest is focused upon three primary problems: (1) what lethals are, (2) how they occur, (3) how, when, and where they act. The third problem comprises a large field of embryological and physiological investigations, but the first and second problems can be approached partially from the cytogenetic standpoint. Light may perhaps be shed on these questions by two lines of study. (a) Comparison of spontaneous and induced lethals, and (b) study of the relationship of genetic lethals to cytological deficiencies. The present paper attempts to give the results obtained from a particular study along these lines.

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Nineteen lethals which had arisen spontaneously were studied. These lethals had been collected over a period of many years by Dr. M. DEMEREC and were maintained in his stocks carried over Inversion dl-49. Thirteen lethals induced by X-ray treatment in experiments by the same investigator and similarly maintained in stocks were taken for comparison. All the lethals used were sex-linked and located to the left of garnet.

METHODS

Since previous work has shown that some of the induced lethals are cytologically detectable, the two investigations mentioned above may be attacked simultaneously by studying the salivary gland chromosomes of mature larvae known by genetic tests to carry lethals which have arisen spontaneously. In a majority of cases the lethal was subjected to the standard crossover tests in order to determine its approximate genetic position:

(1) *l*/dl-49, *y Hw* m^2 g^4 by *ec* ct^6 *v* g^2.

(2) F_1 *l*/*ec* ct^6 *v* g^2 by dl-49, *y Hw* m^2 g^4 and counts were made of all females and males. In other cases as will be discussed later special crossover tests were necessary.

After the location within certain limits was thus known, each lethal was studied cytologically in the salivary gland chromosomes. Preparations were made from mature female larvae. For this purpose the stock females *l*/dl-49, *y Hw* m^2 g^4 were outcrossed to g^2 *B* $\underline{XY}''$ $\underline{YY}''$ males. In the F_1, two types of females are recovered, one being homozygous and the other heterozygous for *g*. The former in the larval stage show white Malphigian tubules and the latter have tubules yellow in pigmentation. By utilizing this difference it was possible to select always the larvae carrying the lethal. Slides were prepared according to the aceto-carmine method and were made permanent by the alcohol-euparal technique described by BAUER (1936). For studying the material use was made of a Zeiss 90×, 1.4 N.A. apochromatic objective, and oil immersed achromatic 1.4 N.A. condenser, compensating oculars (10 and 12.5×), a Bausch and Lomb research lamp (ANTHES 1936; BRIDGES 1936) equipped with a Wratten filter No. 62.

Both synapsed diploid and unsynapsed haploid chromosomes were studied, in every case using only good and well-stretched figures. Each determination was of necessity checked by other persons in the laboratory, including C. B. BRIDGES, M. DEMEREC and B. P. KAUFMANN. The fact that in some cases deficiencies were not detected does not constitute definite proof that deficiencies do not exist in that particular material. Negative evidence on such a point is not conclusive. Furthermore, it should be mentioned here that no attempt was made in the scope of this work to

make exact determinations as to the limits of each deficiency. It was considered sufficient for the present purpose to determine the occurrence of a deficiency without pursuing a more specialized study.

RESULTS

In table 1 is given a summary of the genetical and cytological data for the 32 lethals studied. They are listed in order of location from left to right of the chromosome. Cytological deficiencies are noted in all cases where they were detected; each analysis was verified as noted by several investigators. The reference system is to BRIDGES' (1935) salivary gland chromosome map except in some instances when Dr. BRIDGES furnished a revision of certain regions. The genetic location, as already mentioned, was determined by crossing over tests for which the data are presented in detail. Several special crosses were made:

291-44 *l/rb cx*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—288; *cx*—4; *rb*—24

This shows that the lethal lies between *rb* and *cx* in the ratio 24:4 corresponding to the locus 8.4.

291-3 *l/rb cx*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—485; *cx*—30; +—6

This shows that the lethal lies to the right of and close to *cx* at approximately 14.8.

291-7 *l/t*^{2} *v f*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—207; *v f*—2; *t*^{2}—7; *t*^{2} *v*—51

This shows that the lethal lies between *t* and *v* in the ratio 2:7 corresponding to the locus 28.7.

291-9 *l/ct v dy g f*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—936; *ct v dy*—62; *v dy*—2

This shows a slight reduction in crossing over between *dy* and *g* and considerable reduction between *ct* and *v*. No chromosomal aberration is present but it is possible that in addition to the deficiency in 8C, bands may be missing in 7D.

291-11 *l/ct v dy g*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—705; *v dy g*—88; *dy g*—33; *g*—4; *ct v dy*—64; *v dy*—2

This shows that the lethal lies between *dy* and *g* in the ratio 66:4 corresponding to the locus 36.7. Salivary gland chromosome analysis here led to the suspicion of a very minute inversion and this possibility is not excluded.

291-53 *l/ct v dy g f*×dl-49, *y Hw m*^{2} *g*^{4}

♂♂: non-crossovers—199; *v dy g f*—23; *dy g f*—20; *g f*—5; *f*—3; *ct v*—2; *ct v dy*—4; *ct v dy g*—28; *wings-folded*—9.

TABLE 1

Summary of genetical and cytological data for spontaneous and induced lethals.

NUMBER	DEFICIENCY FOR	LOCATION	*ec* *ct* *v* *g*	*ec* — — —	*ec* *ct* — —	*ec* *ct* *v* —	*ec* *ct* *v* *g*	— — *v* *g*	— — *v* *g*	— — — *g*	*ec* *ct* *v* *g*	— *ct* — —	— *ct* *v* —	— — *v* —	*ec* — — *g*	*ec* — — *v*	LETHAL LIES BETWEEN IN RATIO
		Spontaneous															
291-54	— —	left of *ec*	33	4	7	7	—	—	—	—	—	—	—	1	1		
-68	4A4*	7.0	364	61	18	4	7	—	1	—	—	—	—	1	—		62: 7; see text
-44	— —	8.4															
-100	5C4·5*	*ec-*	40	6	4	9	—	—	—	—	—	—	—	1	—		
-3	5D3·4†	*ec-ct*	85	6	12	13	13	—	—	—	—	—	—	—	1		see text
-95	— —	*ec-ct*	77	5	2	6	—	—	—	—	—	—	—	—	—		
-82	— —	18.8	50	1	14	9	10	—	—	—	—	1	—	—	—		1:11
296-14	— —	21.0	108	—	12	15	19	1	—	—	2	—	—	—	—		1:12
-15	— —	22.0	95	—	16	17	12	2	—	—	—	—	—	—	—		2:16
-8	— —	23.9	109	—	11	22	26	6	—	2	3	1	—	—	—		6:14
-13	— —	24.3	67	—	4	5	13	2	—	—	1	—	—	—	—		2: 4
-19	— —	25.2	83	—	12	6	20	8	—	—	2	—	—	—	—		8:12
291-69	7E9·10†"	27.2	68	—	3	27	23	18	—	5	1	—	—	—	—		18: 8
-7	in8B†	*ct-v*	24	—	4	13	15	17	—	—	2	—	—	—	—		see text
-9	in8C*	37.0	66	—	—	6	14	15	4	—	1	—	—	—	—		4: 6; see text
-11	in12E*	*ct-v*	73	—	—	8	7	5	4	—	2	—	—	—	1		see text
-53	in13B*	40.8															see text
296-22	— —	41.4	165	—	—	7	20	27	22	—	—	—	1	—	—		22: 8
291-5	14B6*	right of *g*	69	—	—	—	13	13	13	—	—	—	—	1	—		see text
		Induced															
294-44	— —	*ec*	69	9	15	7	—	—	1	—	—	—	—	2	—		
302-1	— —	*ec*	37	11	9	9	—	—	—	1	—	—	—	1	—		
-9	— —	7.0	75	13	8	7	2	—	—	1	—	—	—	1	1		2:15
-11	4A1·2†	7.7	28	11	5	5	2	—	—	—	—	—	—	—	—		2:11
304-69	in4C*	10.3	156	14	7	9	7	—	—	1	—	—	—	—	—		7:14
302-3	— —	10.3	156	16	12	3	—	8	—	—	—	—	—	—	—		8:16
294-22	— —	15.2	108	6	17	23	12	—	—	—	1	1	—	—	—		14:6
-27	— —	25.0	80	—	5	13	10	3	—	—	1	—	—	—	—		3: 5
304-71	— —	26.0	186	—	16	15	44	18	—	—	—	1	—	—	—		18:17
294-16	10B8*	33.5	111	—	—	21	25	12	1	—	2	—	—	—	1		1:23
-31	in10C*	33.7	55	—	—	15	19	22	1	—	3	—	—	—	—		1:18
304-68	— —	34.1	51	—	4	10	19	16	1	—	1	—	—	—	—		1:11
-60	— —	43.0	67	—	—	1	16	20	14	—	—	—	—	—	—		14: 1

* Analysis verified by M. Demerec and B. P. Kaufmann.
† Analysis verified by C. B. Bridges, M. Demerec and B. P. Kaufmann.

This shows that the lethal lies between *dy* and *g* in the ratio 4:5 corresponding to the locus 40.8. The "lethal" males which also carry a cytologically detectable deficiency will come through under excellent food conditions. These males have folded wings and are sterile and very weak.

291-5 l/g^2 un^3 f × dl-49, y Hw m^2 g^4

♂♂: non-crossovers—199; g^2—27

This shows that the lethal lies to the right of and very close to *g*.

The cytological evidence is summarized in the chart shown in figure 1. In this chart the location of induced and spontaneous lethals is indicated both on the linkage map and on the salivary chromosome map and drawings of critical salivary chromosome figures are shown. In summarizing the cytological data of 19 spontaneous lethals, 9 or 47.3 percent are detectable deficiencies. Among the 13 induced lethals 4 or 30 percent are deficiencies. This value agrees fairly well with the 40 percent obtained by SAKHAROV (1935) and ALIKHANIAN (1937).

DISCUSSION

It would seem impossible to escape from the fact already mentioned that in any consideration of lethals a whole range of types of changes presents itself. The present work also provides evidence that genetic lethals may or may not be detectable cytologically. Whether this is because such minute deficiencies are undetectable with available facilities is not certain. Possibly some changes in genes may so affect their physiological activity as to produce a genetic lethal effect, whereas the activity of that gene as expressed in its ability to be represented by nucleic acid production in the form of a salivary chromosome band remains unaffected. In other words, the various lines of activity of a gene are differently sensitive to the environment. Cases are already known of cytological deficiencies having no genetic effect (DEMEREC and HOOVER 1936), just the reverse of the above condition. The hypothesis may be offered that all mutations, deficiencies, and all other types of change are merely expressions of the differential response of the various lines of activity of the genes and that accordingly all these types could be classified and arranged in a more or less orderly series. Be that as it may, evidence is available here that many of the genetically detectable lethals are cytological deficiencies. It is significant that this is true of both spontaneous and induced lethals, and that in this respect the two types of lethals do not differ. This stands in contradiction to the suggestion sometimes made that induced lethals are separable in type from the spontaneous ones. So far as it is known there is no difference between the spontaneous and induced lethals which have been studied in known loci. For example, in the Notch region where a total of 12 lethals were studied by SLIZYNSKA (1938) the

two spontaneous ones presented no evident distinction as to size of deficiency or its effect. Some of the gene changes produced by X-ray treat-

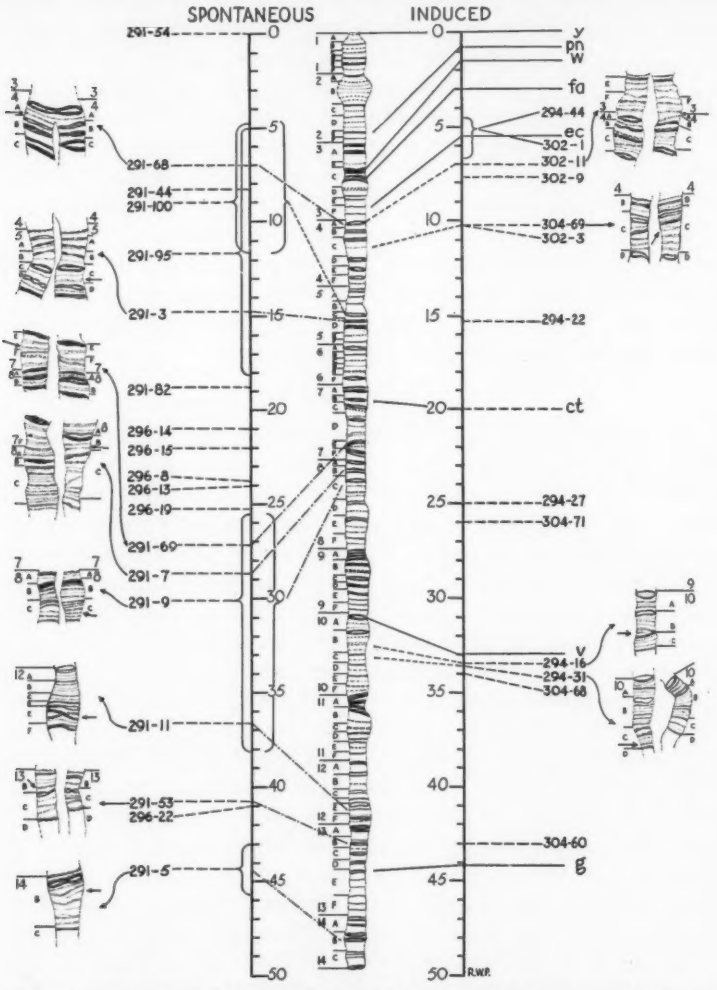

FIGURE 1. Chart indicating location of studied lethals on the linkage map and when known on the salivary chromosome map. Critical figures indicating deficiencies are shown for certain lethals.

ment are in every respect identical with those obtained spontaneously. Similarly here the indications are that induced and spontaneous lethals are alike.

DEMEREC (1937) has suggested that when the frequency of deficiencies is low they would not be expected to be connected with chromosomal aberrations, and that the proportion of aberrations would necessarily increase with an increase in the frequency of deficiencies. Hence the failure of available data to indicate an association between chromosomal aberrations and spontaneous lethals is explicable because the frequency of spontaneous lethals is too low. If this is true, another difference noted between spontaneous and induced lethals is removed.

The results of the present work are not in agreement with those of SAKHAROV (1935, 1936), who studied 27 lethals induced by X-ray treatment and 25 spontaneous lethals all sex-linked. Among the induced lethals, SAKHAROV found 17 chromosomal aberrations. Among the other 10 he found 4 instances of deficiencies. Among the spontaneous lethals he found no instances at all of aberration or deficiency. SAKHAROV and NAUMENKO (1936) also reported studies of lethals produced by treatment with iodine and manganese and again no cases of cytological disturbance were found. The conclusion is consequently drawn that spontaneous and chemical lethals are of a different type than those induced by X-ray treatment. Since in the results recorded here a minimum of 16 percent of spontaneous lethals and deficiencies was found, the conclusions above do not fall into line.

It may be noted with reference to the chart in figure 1 that lines drawn to connect the approximate loci of the lethals or known loci on the cytological map with similar loci on the genetic or crossing over map are not always parallel.

SUMMARY

Nineteen cases of spontaneous lethal changes in the X chromosome of *Drosophila melanogaster* were studied. Their genetic locations were determined by standard crossover tests and their cytology studied in salivary gland chromosomes. Of these, 47.3 percent were found to be minute deficiencies. Among the 13 induced sex-linked lethals studied for comparison 30 percent were detectable deficiencies. These results would seem to indicate that there is no primary distinction between spontaneous and induced lethals.

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SALIVARY CHROMOSOME ANALYSIS OF THE WHITE-FACET REGION OF DROSOPHILA MELANOGASTER

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THE white-facet region of the X chromosome has been utilized extensively in both genetic and cytological studies. It is interesting genetically because of the unique series of alleles recognizable in the white locus and because of the series of alleles and the dominance of the deficiency for the facet locus. It is interesting cytologically also because of the grouping of heavy bands visible in salivary gland chromosomes. In this paper, the results of genetical and cytological studies of fourteen deficiencies in that region are reported.

Except for N-8 (MOHR 1923), 264-36, 264-37, 264-38, and 264-39, the material for all other deficiencies and the data for a number of genetic tests were obtained from M. DEMEREC. N-8 (MOHR) and 264-38 originated spontaneously while all other deficiencies were induced by X-ray treatment. Critical salivary chromosome figures were checked by C. B. BRIDGES, M. DEMEREC and B. P. KAUFMANN. Photographs and redrawing of figures were done by Miss R. W. PARKER.

METHODS

In order to obtain Notch deficiencies, normal males carrying yellow (*y*—0.0) as a marker were irradiated with approximately 2500 r-units, and then were mated with females homozygous for cherry (w^{ch}—1.5) and wavy (*wy*—40.7). From F_1 females the white or Notch flies were selected for this work.

H. BAUER'S (1936) method for preparing salivary glands was followed. Full grown larvae were dissected in aceto-carmine; needles and forceps of stainless steel were used to avoid contamination by iron. Slides were made permanent by the alcohol-euparal technique. Microscopical observations were made using a 2 mm. 1.4 N.A. apochromatic objective, an oil immersed achromatic 1.4 condenser, and 12.5× or 15× compensating oculars. A Bausch and Lomb research lamp equipped with a green Wratten filter No. 62 was used as a source of light. Drawings were made with the aid of a camera lucida.

Non-synapsed chromosomes were selected for study, because even good preparations of the region in question usually show such distortion that identification of bands is very difficult. In non-synapsed chromosomes the number of bands involved was determined and the length of the deleted

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part was found by comparing the deficiency with a normal haploid strand. In many cases the bands of this region were identified easily by their characteristic size and shape, as well as by the intensity of their staining. To reduce any possibility of error, however, the limits of the deficiency were determined also in the synapsed condition of the chromosome. In that case, the exact position of some deficiencies was determined by combining them with the 258-18 translocation, in which the tip of X chromosome to 3C4 inclusive is transferred to the spindle fibre region of the fourth chromosome, exchanging places with the remainder of the fourth (DEMEREC and SLIZYNSKA 1937). This gave a clearer picture of synapsis in the one end of the deficiency and at the same time it usually prevented synapsis at the other end. But all these methods of examination sometimes failed, especially when the studied band was very faint and lay in a very narrow space between two heavy darkly stained bands or vesicles.

RESULTS

The results of both breeding and cytological studies are summarized in figure 1. For a detailed description, BRIDGES' salivary gland chromosome map and his reference system (BRIDGES, 1938) were used. According to this system, every vesicle is counted and numbered as two separate bands.

258-11. This deficiency, as shown by breeding results, includes only one known locus, white, and extends in its cytological picture from 3A3 to 3C2.3 inclusive. In this case it is impossible to determine whether the 3C4 band is present or not, because this band is so faint that it is rarely visible even in the normal chromosomes. It was seen only twice during this work. 258-11, therefore, involves fifteen or sixteen bands.

258-14. Genetically identical to the former, this deficiency is a little shorter cytologically. The first band involved in the deficiency is 3A4, and the last band deficient without any doubt is 3C1. However, the possibility that 3C2 is also absent has not been excluded.

N-8 (Mohr). Cytological data in regard to this deficiency differ in some respects from those which are given by MACKENSEN (1935) and GOTTSCHEWSKI (1937). According to MACKENSEN, this deficiency does not involve 3C1, but involves the double band 3E1 and 3E2. On the contrary, GOTTSCHEWSKI shifts the limits to the left and places the deficiency from 3C1 to 3D4 inclusive. According to the data presented here, the deficiency extends from 3C1·2 to 3D5·6 inclusive. This determination is based on studies of both synapsed and non-synapsed strands. In haploid chromosomes with N-8 deficiency, between section 3B and 3E, only one dark band is present which may be 3C1 as well as 3E1·2. Although the general features of the band in question seemed to indicate the second possibility, additional preparations were made with the translocation 258-

18, to determine accurately the homology of this band. As is evident from the drawing and from the photomicrograph in figure 2c and 2d, the band in question synapses with one lying in the part of another haploid strand not affected by the translocation, namely, with 3E1 · 2. This gives critical evidence for limiting the size of N-8 deficiency to the dimensions stated above. Therefore, the N-8 deficiency includes 18 bands.

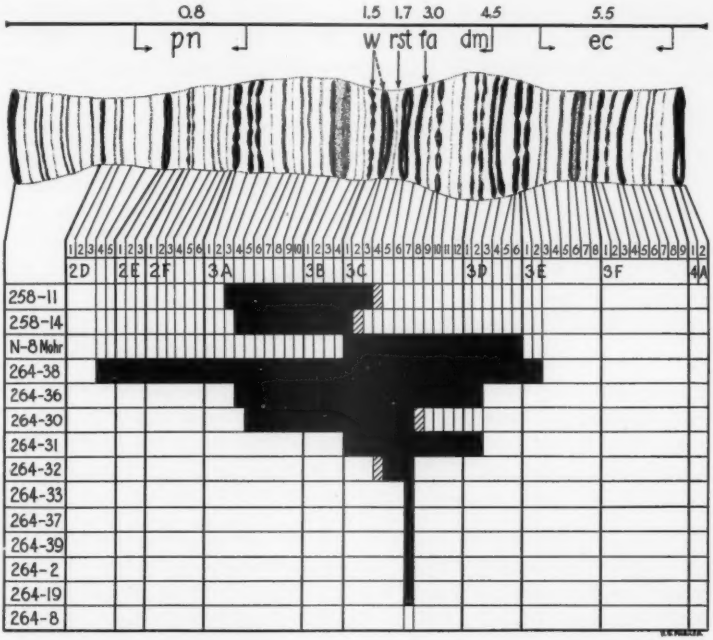

FIGURE 1. Genetic and salivary chromosome maps of white-Notch region of the X chromosome and a diagram showing results of the study of fourteen deficiencies of that region. Black areas represent deficient segments and shaded areas indicate sections for which it is uncertain whether or not they are deficient.

264-38. This is the largest deficiency described in this paper; it involves 45 bands, from 2D4 to 3E1 · 2 inclusive, and according to breeding results involves the loci *pn*, *w*, *rst*, *fa*, and *dm*. The females heterozygous for this deficiency show greatly reduced viability as compared with all other Notch cases, and even in good culture conditions they are distinctly smaller than other females. The drawing and photomicrograph figures 2k and 2l show the 264-38 deficiency as it appears in diploid condition, with the small loop formed by the normal haploid.

264-36. This deficiency involves the loci *w*, *rst*, *fa*, and *dm*. Cytologically it covers the section from 3A4 to 3D2 inclusive, containing 25 bands. A drawing and a photograph of that deficiency in a heterozygous condition is shown in figures 2a and 2b.

264-30. The photomicrograph in figure 2j shows a haploid strand of this deficiency. As is indicated on the corresponding drawing, figure 2i immediately following the group of dark bands 3A1 to 3A4 is a much lighter dotted line 3C9·10, not clearly visible on the photograph. The band 3C8 is probably present but since it was seen only in oblique light, this determination was marked as questionable on the diagram in figure 1. The deficiency includes 17 or 18 bands.

264-31. This deficiency, genetically, removes the loci *w* to *dm* inclusive. Cytologically, fourteen bands from 3C1 to 3D2 inclusive are missing.

264-32. This deficiency involves only the *rst* and *fa* loci. The left end starts between 3C2·3 and 3C5·6. The limit in regard to 3C4 is not determined exactly for the same reason as in the case of 258-11. The last band included in this deficiency of 3 or 4 bands is 3C7.

264-37, -39, -33, -2, -19. These five deficiencies are identical with one another in every respect. All were tested with *fa*, fa^n, *spl*, and *Ax* and showed the deficiency for them but not for other adjacent loci. Cytological observation disclosed in all these cases a short deficiency for only one band, 3C7. On photomicrograph figure 2f and drawing figure 2e, 264-39 is represented. The bands 3C1 to 3C5·6 appear as three dark, wide bands. The next band, 3C7, present in the left haploid does not have a corresponding band on the right side. The band 3C8 not well seen on the photograph is again present in both strands and the next two bands (3C9.10), which are clearly visible, are the last synapsed bands on this figure.

264-8. In this case, although breeding tests indicate that *fa*, fa^n, *spl*, and *Ax* loci are deficient, the cytological analysis did not disclose any visible deficiency. In homozygous condition, it produces a lethal effect not only for the whole organism but, according to the data obtained by DEMEREC (1934), it shows a cell-lethal effect in the hypodermal cells of females. The females heterozygous for the 264-8 deficiency show the whole complex of changes characteristic for other Notches. Since the region studied in this case consists of very dark bands usually fused together, the examination of single haploids will show best whether all bands are present. In order to obtain a higher number of non-synapsed strands and to identify the deficient chromosome, N-females of the genetical constitution N/dl-49, *y Hw* m^2 g^4 were mated with males carrying the 258-18 translocation. Of three chromosomes involved in this cross, one contains the dl-49 inversion, another the translocation X-4 so that the identification of the third chromosome carrying the change in the N-region does not

present any difficulty. The photomicrograph figure 2h and drawing in figure 2g, made from the preparation of female larvae from the cross described above, represent the section of a haploid strand of the N-chromosome. It shows clearly that all bands known for this region are present. Moreover, the distance between the single bands and the general appearance of the bands as to size, width, and intensity of staining, do not differ from those which were observed for the normal X chromosome.

DISCUSSION

The cytological and breeding results are summarized on the chart of figure 1. On this chart a close relationship between genetical and cytological pictures of the deficiencies studied is evident, indicating that the deficiencies which are similar in their genetical features always have at least one band in common. This serves as a means for determining the position of various loci in salivary gland chromosomes. In this study the problem is limited to the region of the X chromosome from *pn* to *ec*. This section of the chromosome has also been studied by MACKENSEN (1935), GRÜNEBERG (1937), EMMENS (1937), and GOTTSCHEWSKI (1937). A comparison of the cytological results of different authors, however, presents difficulties because different reference systems are used in different papers.

The limits of the section in which prune (*pn*) is located are given by the left end limits of the 264-38 and 258-11 deficiencies. According to that evidence, the gene is located somewhere between 2D3 and 3A3. MACKENSEN's determination is closer because he designates for this gene the region 2D6 to 2F2 inclusive which probably corresponds on the revised BRIDGES' map to 2D3 or 2D4 to 2F2.

For the location of white (*w*-1.5) evidence was obtained from 258-14, *N-8*, and 264-31 deficiencies. *N-8* and 264–31 place this locus to the right of the 3B4 band, because 3C1 is the first missing band in both these cases. The 258-14 deficiency, which also includes the *w* locus, shows that this gene must be located to the left of 3C3 since this band lies beyond the limits of the deficiency. A number of figures examined during these studies indicate that 258-14 is not deficient for the 3C2 band, and if this is true, the band 3C1 represents the white locus.

According to GRÜNEBERG (1937) roughest (*rst*) is located 0.2 to the right of *w*. The same author states that *rst*^{3} is associated with a long inversion. EMMENS (1937) determined both limits of this inversion, the left one being between 3C2·3 (designated by him 3C2) and 3C5·6 (designated 3C3) and the right in the inert region to the right of *bb*. EMMENS also studied *rst*^{2} and described it as a deficiency for bands 3C4 to 3C7 inclusive. The deficiencies described here throw some light on this question. The deficiency 258-11, which does not include *rst*, shows that *rst* should

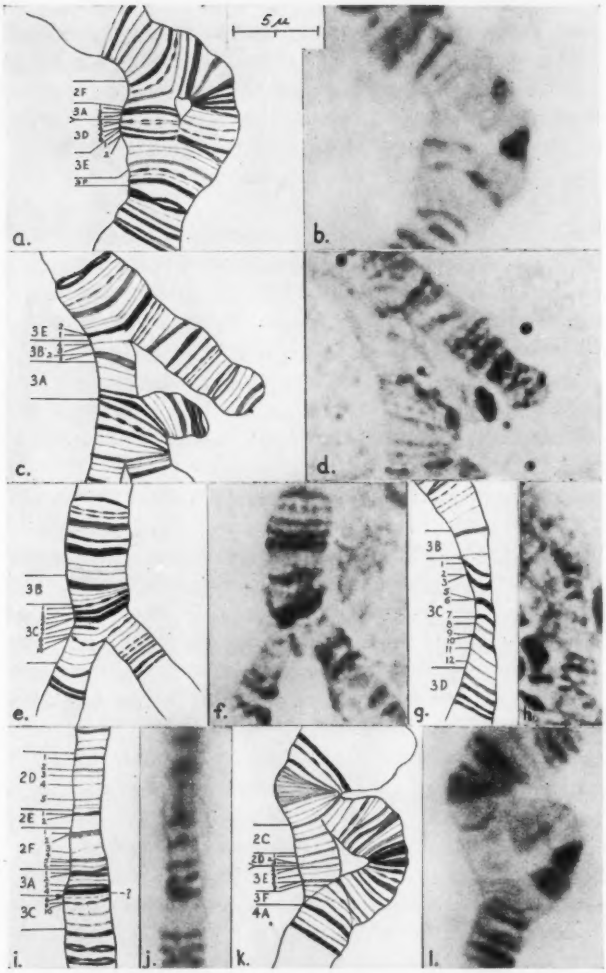

FIGURE 2. Drawings and photographs of various deficiencies. (a) and (b) 264–36 deficiency involving *w*, *rst*, *fa* and *dm* loci and cytologically a section of 25 bands, from 3D4 to 3D2 inclusive; (c) and (d) N-8 (Mohr) heterozygous with X-4 translocation 258-18 involving 18 bands, 3C1·2 to 3D5·6; (e) and (f) 264-38, one band deficiency; (g) and (h) haploid strand of 264-8 showing all bands; (i) and (j) 264-30 deficiency involving 17 or 18 bands, 3A5 to 3C7 or 3C8; (k) and (l) 264-38, the longest deficiency of this series involving 45 bands, 2D4 to 2E1·2.

be to the right of its right end, namely to the right of 3C2·3. The deficiency 264-32, which includes *rst* and *N* according to the genetical data, starts following 3C2·3 and includes 3C5·6 and 3C7, indicating that *rst* is located between 3C2·3 and 3C8. Deficiencies 264-33, -37, -39, -2, and -19 all of which include the 3C7 band but do not include *rst* show that *rst* is located to the left of 3C7, namely that it is represented either by band 3C4 or 3C5·6. Cytological analysis of translocation 258-18 (DEMEREC and SLIZYNSKA, 1937) shows that the break in the X chromosome occurred genetically after *rst* and cytologically between 3C2·3 and 3C5·6. Since 3C2·3 cannot represent *rst*, as shown by the deficiency 258-11, it seems probable that *rst* is represented by 3C4.

Facet, facet-notched, split, and Abruptex loci will be discussed together because their behavior in crosses with Notch is identical. It is known that notches are deficiencies for all these loci. The results of MACKENSEN place the locus for these genes at bands 3C4 to 3C7 or even probably 3C8. EMMENS locates it at 3C9·10, and GOTTSCHEWSKI put the genes *co*, *fa*, *spl*, and *Ax* in bands 3C5, 3C6, 3C7, and 3C8 respectively. The material used for our study includes five cases of notch deficiency, each including only the genes mentioned above. Cytologically they all show a deficiency for only one band, 3C7. If it is kept in mind that all cytologically known deficiencies occupying that band are, genetically, deficiencies for all these loci, it can be assumed that all these are represented by this one line. Therefore, there are two probabilities supported by cytological evidence: either these genes are allelic to each other, or one band covers several loci. Genetical data, however, are in favor of the first possibility.

The locus of diminutive (*dm*) should be placed to the left of the right end of 264-31 which includes it.

MACKENSEN (1935) has placed the position of the echinus (*ec*) locus in the region between 3E2 and 3E4. None of the deficiencies mentioned here includes *ec*, therefore, this locus must lie to the right of the right end of the longest of them, which is the 264-38; its righthand limit follows 3E2.

Notch 264-8 presents an interesting case of a genetical deficiency without any detectable change in the salivary gland chromosome. A similar Notch deficiency was first described by MACKENSEN (1935). To explain this phenomenon he assumed a dominant mutation with a Notch character. A second case studied in more detail was the Notch-G case described by GOTTSCHEWSKI (1937), who states that cytological examination showed a normal condition in the notch region. To explain the case, GOTTSCHEWSKI discusses three possibilities. First is the chain mutation hypothesis wherein gene changes have occurred in all the involved loci, producing allelomorphic forms, which, when crossed with respective recessive genes, give the illusion of pseudodominancy. Since this possibility requires several in-

volved assumptions he does not think it is probable. As a second possible explanation for removing the genes without the destruction of normal banding he suggests that some other agent might be responsible for the bands, which also seems improbable. He assumes, as the best explanation, the working hypothesis of inactivation, wherein the gene, although physiologically inactive, does not prohibit the formation of the band. For the present, no better explanation of the problem discussed here seems available.

Among the deficiencies studied, two are spontaneous and twelve induced. One of the spontaneous, 264-39, involves a single band; the other, N-8 Mohr, a block of 18 bands. Five induced deficiencies involve only one band while the remaining six cover larger sections. Although the number of cases studied is small, it appears that spontaneous deficiencies are similar to induced.

If the crossing over map for the region studied is compared with the approximate location of the genes in salivary chromosomes, an interesting fact may be observed. The distances between genes on the standard map do not correspond with those in salivary chromosomes. For example, the distance between *y* (0.0) and *w* (1.5) is the same on the crossing over map as that between *w* and *fa* (3.0). In the salivary gland, however, *y* is located at the tip of the X chromosome (M. DEMEREC and M. E. HOOVER, 1936) and *w* within the bands 3C1 or 3C2, so that the section limited by them is at least 12 times longer than the section between *w* and *fa* (3C7). It is difficult to determine what is responsible for this discrepancy. Between *y* and *w* there are about 75 bands, and between *w* and *fa* there are only about 5. The relation, therefore, is 15:1. The number of bands between *y* and *pn* is 57, if counted to the middle of the region ascribed for this locus, and the crossing over distance between *y* and *pn* is 0.8; between *pn* and *w* there are 18 bands and 0.7 crossing over units; between *w* and *rst* there are two bands and 0.2 crossing over units; between *rst* and *fa* there are two bands and 1.3 crossing over units. If we now calculate how many crossing over units correspond to one band in each segment separately we will obtain for *y-pn*, the value 0.014; for *pn-w*, 0.038; for *w-rst*, 0.1; and for *rst-fa*, 0.65. It is striking that the numbers are constantly increasing toward *fa*.

SUMMARY

Salivary chromosome studies of fourteen deficiencies affecting *w* and *fa* loci were made. The results are summarized in figure 1.

An approximate determination of the position on the salivary chromosome map for the loci involved was made. The locus *pn* (0.8) is placed between 3D4 and 3A2; *w* (1.5) is represented by 3C1 or 3C2; *rst* (1.7) by

3C4; *fa*, *spl*, and *Ax* (3.0) by 3C7; *dm* (4.5) is placed between 3C9 and 3D2 inclusive; and *ec* (5.5) to the right of 3E2.

One case of genetical deficiency without any visible effect in salivary glands chromosome structure is described.

The relations between the crossing over map and the salivary chromosome structure are discussed.

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THE INDUCTION OF OÖGONIAL CROSSING OVER IN DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

OÖGONIAL crossing over may be defined as homologous exchange between chromosomes in oögonial cells. Its existence has been suspected because of the similarities between somatic crossing over, induced increases in crossover values in females, the recently demonstrated spermatogonial crossing over in males (FRIESEN 1936, STERN and DOAN 1936, WHITTINGHILL 1937) and also because of the simultaneous appearances of rare crossovers in untreated females (SEREBROVSKY 1927). The similarities are that recombinations are produced or increased by the same agents, high temperatures and X-rays, and that the recombinations are found clustered in the descendent body tissues or offspring.

Recognition of oögonial crossing over by means of like recombination offspring is difficult because the individual cells of such groups are variously altered by the numerous, subsequent meiotic exchanges and are thereby distributed among many recombination classes. If these regular meiotic exchanges could be suppressed in females, as they normally are in males of Drosophila, without using such chromosomal irregularities as inversions and translocations, oögonial crossing over might thereby be unmasked. Such an agent is available in the recessive asynaptic factor, c3G, found by GOWEN and located in the third chromosome group.

The action of c3G on crossing over in the different chromosomes is of special interest. GOWEN (1933) found a very low frequency of crossing over: about 1 per 1000 between *st* and *ca* in chromosome III and between *al* and *sp* in II, and about 1 in 1800 for the first three regions of the series *sc ec ct v g f* in the sex chromosomes. These crossovers were for regions away from the spindle attachment in all cases except half of those in chromosome III. Although meiotic crossing over is almost entirely inhibited, somatic crossing over, revealing itself in the "mutant" spots recorded earlier by GOWEN (1929), seems to be unaffected. Hence with respect to crossing over c3G females are comparable to normal males of *Drosophila melanogaster*. If oögonial crossing over could be induced in the former, it would be as easily detectable as spermatogonial crossing over has been in the latter.

[1] Experiment carried out while holding a National Research Council Fellowship in Biology at the California Institute of Technology.

The writer is indebted to the National Research Council for the opportunities of a year's fellowship, during which this work was performed, and to members of the Kerckhoff Biological Laboratories of the CALIFORNIA INSTITUTE OF TECHNOLOGY for suggestions and criticism of methods and of interpretations.

EXPERIMENTAL PROCEDURE

It was planned to backcross, after treatment with X-rays, females heterozygous for alternated "X^9" but homozygous for c3G to the multiple recessive "X^9" males; but in the course of making up the stocks not all of the mutants *sc cv v f bb* and *ec ct*^{6} *s car* were retained in all the stocks used. However, all F_1 females were marked with *v f/car* as a minimum.

After irradiation by 2500 r the F_1 females were mated individually in vial cultures to several "X^9" males. All were changed to four successive cultures, and more males often added, at the end of 6, 10, 14 and 18 days from the time of X-raying, whether fertile or not. Cultures were kept at 25°C and were examined finally on the 17th day. All crossovers were verified by mating again to "X^9."

RESULTS

Among 10,826 offspring 16 recombinations grouped in only 11 of the 133 families were found. The crossovers and total offspring of these 11 treated females are given in table 1 by cultures. One female, g, proved to have been a triploid, the only one found in the F_1. Two females, a and d, were definitely XXY, as shown by the viability of one or more patroclinous males, which carried *bb*^{l} in the X. For the same reason at least 11 other females which did not produce any crossovers must have been XXY. The remainder, 9 and 111 from the crossover and non-crossover-producing groups, respectively, did not definitely show the presence of an extra Y, for although matroclinous offspring were numerous in many families, they were more probably primary exceptions due to the action of c3G on disjunction.

An index of the gonial origin of a majority of the crossovers was found (table 1) in the pronounced grouping of the recombination classes. Of all the recombinations for the *ec-ct* interval, three were found in one family, the other in a second. For the *cv-ct* interval in more fully marked females, two of the crossovers, which were reciprocals, were found in one family, the other elsewhere. In the next region, *ct-v*, the six crossovers were distributed among only five progenies. In the *s-f* region, as in that between *cv* and *ct*, only 3 crossovers were found among the progeny of 133 females, yet 2 of them occurred in one family. Clearly these clustered crossovers cannot be accounted for by independent occurrences of crossing over in

TABLE 1

Crossover-producing families.

	COMPOSITION OF F_1 c3G FEMALES	RECOMBINATION CLASSES	Successive cultures→ / Days after x-raying→	1st 0–6	2nd 6–10	3rd 10–14	4th 14–18	5th 18–23	TOTALS
				TOTAL RECOMBINATIONS / TOTAL OFFSPRING					
a.	$\frac{v\ f}{ec\ ct^6\ car}$ Y	$ct^6\ car$				2	1		3
				25	48	60	57	—	190
b.	$\frac{v\ f}{ec\ ct^6\ car}$	$ct^6\ car$			1				1
				13	20	30	27	24	120
c.	$\frac{sc\ cv\ v\ f}{ec\ ct^6\ s\ car}$	$sc\ cv\ ct^6\ s\ car$					1		1
		$ec\ v\ f$ Haplo-*IV*						1	1
				—	—	49	42	45	136
d.	$\frac{sc\ cv\ v\ f}{ec\ ct^6\ s\ car}$ Y	$sc\ cv\ ct^6\ s\ car$		1					1
				55	—	29	26	9	119
e.	$\frac{v\ f}{ec\ ct^6\ car}$	car				1	1		2
				—	—	36	26	3	65
f.	$\frac{sc\ cv\ v\ f}{ct^6\ s\ car}$	$sc\ cv\ s\ car$						1	1
				—	19	19	2	3	43
g.	$\frac{v\ f}{sc\ cv\ v\ f}$	$sc\ cv\ car$			1				1
	$ec\ ct^6\ car$			—	50	14	—	—	64
h.	$\frac{v\ f}{ct^6\ s\ car}$	$s\ car$					1		1
				—	—	30	50	39	119
i.	$\frac{v\ f}{ec\ ct^6\ car}$	car					1		1
				—	—	—	30	14	44
j.	$\frac{v\ f}{ct^6\ s\ car}$	$ct^6\ s\ f$				1	1		2
				57	66	43	68	3	237
k.	$\frac{v\ f}{ct^6\ s\ car}$	$ct^6\ s\ f$				1			1
	(in mass cultures)			252	317	387	319	216	1491

meiosis. Rather each cluster of recombinations (table 2) should be attributed to a single occurrence of gonial crossing over in a single female.

TABLE 2

Crossovers and crossing over by region and family.

REGION	FAMILIES IN WHICH CROSSOVERS OCCURRED: SINGLY	DOUBLY	TRIPLY	TOTAL	TOTAL CROSSOVERS
ec–ct	1		1	2	4
cv–ct	1	1		2	3
ct–v	4	1		5	6
s–f	1	1		2	3
Totals	7	3	1	11	16
Non-crossover families and flies				122	10,810

TABLE 3

Crossovers in relation to time of X-raying.

CULTURES Days after X-raying	1st 0–6	2nd 6–10	3rd 10–14	4th 14–18	5th 18–23	TOTALS
Oögonial crossovers	—	—	4	4	1	9
Possibly meiotics	1	2	1	2	1	7

The gonial origin of these multiple occurring crossovers was further indicated by the time of their appearance (table 3). If all induced crossing over occurred during the time of exposure to X-rays, the recombinations produced in the younger cells would appear in the later hatches of offspring. It is significant that the clustered crossovers did not appear in the earlier cultures but did appear in those started ten or more days after X-raying. Although there were but few crossovers, they apparently took longer to appear than those induced by PLOUGH (1917) and by MAVOR and SVENSON (1924) in females and as long as the more pronounced spermatogonial crossovers found by FRIESEN (1936). On PLOUGH'S calculations, all cells which were oöcytes when X-rayed would have been laid before the tenth day, which is the earliest that one of these oögonial crossovers could have been deposited as an egg.

A third check on the stage of development at which our crossovers were produced was the distribution of mutations. Some occurred singly; others were found bunched and always in the third and fourth cultures. Scute alleles, with normal salivary chromosomes, were found in two families, in one of which it had been transmitted to eight offspring. Autosomal Minutes appeared in different families in one and five offspring. Two raspberry mutants were found in one family when they were tested first as *v f car* crossovers. A dominant notching of the wings and a suppressor of forked (to be described below) appeared once each. Use of induced mutations has previously been made by HARRIS (1929) to show that male germ cells treated during late stages of their development are used up as sperm within 12 days, after which time the active sperm are derived from cells treated in spermatogonial stages. The gonial origin of such random changes as three of these mutations is hardly open to question. Consequently, the mutations serve as measures of what cells were gonial at the time of treatment. Only the fact of spontaneous crossing over in c3G females prevents the ascribing of all the recombinations of the last three cultures to oögonial exchanges.

The possible gonial nature of some or all of the balance of the recombinations may be obscured in at least two ways. Gonial crossing over may occur too late for its products to include two adjacent eggs and the inter-

vening nurse cells, or it may occur in a female whose offspring are few in comparison with the number of egg strings in her ovaries. The first possibility is difficult to investigate, but the effect of size of family upon the recovery of several crossovers of gonial origin may be indicated in table 4.

TABLE 4

Distribution of crossovers among families of different sizes.

NO. OFFSPRING PER FAMILY	NO. FAMILIES	CROSSOVERS FOUND	
		SINGLY	PLURALLY
1– 25	31	—	—
26– 50	20	2	—
51– 75	27	1	1
76–100	12	—	—
101–125	13	4	—
126–150	11	—	1
151–175	6	—	—
176–200	5	—	1
201–225	2	—	—
226–250	6	—	1

Total families: 133. Average number of offspring: 81.4.
Median number of offspring: c. 65.

Most of the oögonial crossovers were found in families larger than those in which the solitary ones were recovered, and all of the clustered ones were in families larger than the median size. Conversely, three of the singly occurring recombinations appeared in families of 64, 44, and 33 offspring. These numbers were barely large enough to include two eggs from each of the egg strings, of which BERGNER (1928) reports about 30 per normal female.

Although the distribution of crossovers in this experiment was different from that found by GOWEN (1933), the frequency of exchange was about the same. Our frequency of recombinations in the X chromosome was 1/677 and the estimated frequency of crossing over was 1/985. GOWEN'S frequency of recombination (and of crossing over) was 1/612 without the use of X-rays.

Several interesting classes other than recombinations appeared in our experiment, due mainly to the well known effects of the asynaptic factor. Among the 10,000 offspring 158 triploids, 32 haplo-IV flies including one crossover, 10 intersexes, 3 mosaics and 2 gynandromorphs were recorded. One of the last was triploid and wild type on the head and one side and intersex and *sc cv f* on the other half of the thorax and abdomen. The other was a matroclinous female which had lost one sex chromosome and was *ec ct car* male on the head and one-half of the body.

A new mutation named "suppressor of forked" and given the symbol *su-f* was found while verifying what appeared to be a double crossover vermilion fly. It has been located a little to the right of carnation by crossover tests, and the salivary chromosomes appear to be normal in this region. The phenotype of *f su-f* males and of homozygous females often shows a trace of forked on one bristle, like the character hooked.

DISCUSSION

In order to relate this new kind of crossing over to the several other kinds, some of which are also but recently recognized, a classification of them is deemed advisable. One is presented in table 5 in which current terminology is used as far as possible. The term somatic crossing over is reserved in its restricted use for the process when it occurs in the soma, and another term, mitotic crossing over, is proposed to include both the somatic and germinal (spermatogonial and oögonial) exchanges taking place before the maturation divisions. These three kinds of mitotic crossing over are in contrast to the well known meiotic, or oöcytial, exchanges.

TABLE 5

Classification and comparison of different kinds of crossing over in Drosophila melanogaster.

KINDS	MITOTIC			MEIOTIC
	SOMATIC	SPERMATOGONIAL	OÖGONIAL	OÖCYTIAL
Spontaneous occurrences	rarely	rarely (X and Y)		customarily
Frequency altered by	X-rays high temperature Minute factors	X-rays high temperature	X-rays	X-rays low and high temperatures age
Region of greatest change	spindle attachment	spindle attachment		spindle attachment

Thus the primary classification is made upon the basis of the division process involved, and the subdivisions are based upon the cells in which crossing over occurs. That this is a natural classification is brought out by the comparisons included in table 5, where provisionally all the information about germinal crossing over in normal females, treated or untreated, is considered to be entirely due to meiotic crossing over.

From the comparisons of different kinds of crossing over there are indications that the results which have in the past been obtained by subjecting normal females to X-rays or to temperature extremes may be due to crossovers from two sources, gonial and meiotic, rather than only

from the latter. The crossovers induced in normal females resemble the spontaneous meiotic crossovers less than they resemble those induced in somatic or spermatogonial cells. This is true in respect to the regions affected, the time of formation and the activating agents. If further studies on c3G females should show that oögonial crossing over is most frequent in the vicinity of spindle attachments, then it would be reasonable to suppose that a few occurrences of crossing over in early oögonial cells are responsible for most of the increased percentage of recombination offspring. Spontaneous crossing over in oögonial cells might also be the explanation of the unusual distribution exhibited by certain rare crossovers, different classes in different progenies, as noted by SEREBROVSKY (1927), BRIDGES and others.

SUMMARY

1. A 2500 r dose of X-rays does not affect the action of the asynaptic factor, c3G, on meiotic crossing over between the sex chromosomes of females.

2. In oögonial cells crossing over may occur during irradiation of c3G females as shown by the appearance of recombination offspring in unusual clusters from eggs laid ten or more days after treatment. Similarly, groups of identical mutations accompany the clustered crossovers temporally.

3. A classification of the kinds of crossing over into meiotic and mitotic, the latter divided into somatic, spermatogonial and oögonial crossing over, seems justified by the likenesses and differences which are pointed out among them.

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TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 9

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INTRODUCTION

THE *C wx* linkage group associated with chromosome 9 was one of the first to be established in maize. This was largely due to the excellence of the two genes *C* and *wx* as working tools. EMERSON, BEADLE and FRASER (1935) list 28 genes in this group. Nearly all of these are to the left of *wx* or within 10 or 15 units to the right. MCCLINTOCK (1930, 1931), CREIGHTON and MCCLINTOCK (1931), CREIGHTON (1934) and BURNHAM (1930, 1934a, b) have shown that the gene yg_2 is near the end of the short arm of chromosome 9 and *wx* nearer the spindle attachment but still on the short arm. Virescent$_1$ (v_1) is probably on the long arm not far from the spindle attachment (BEADLE 1932, BURNHAM 1934b). The greater portion of the long arm is conspicuous by its lack of definitely known genes. This lack of known genes may be largely due to the difficulty of detecting genes in the distal part of the long arm by means of ordinary linkage tests with such genes as *C*, *sh* and *wx*. Or it may be that such regions are redundant sections of chromosome materials which are also represented elsewhere in the chromosome complement.

Chromosomal interchanges or reciprocal translocations are excellent tools for the exploration of portions of chromosomes where no known genes are available. For most cases it is only necessary to cross any new or unplaced gene with the appropriate translocation stock, backcross with the new gene if recessive or with a normal stock if dominant. Classification of the gene character under consideration and semisterility will give a direct linkage test with a known point on the chromosome.

The chromosome 9 translocations studied by the writer have all been on the long arm, mostly in the region beyond the present known genes. They do not add appreciably to our knowledge of the region covered by the present linkage maps, but do furnish excellent means for the placement and mapping of genes in the extensive region of the long arm where no genes are as yet available.

PREVIOUS DATA ON TRANSLOCATIONS INVOLVING CHROMOSOME 9

T8-9a (BURNHAM 1930, 1934b; MCCLINTOCK 1930, 1931; CREIGHTON and MCCLINTOCK 1931; BEADLE 1932). The interchange on chromosome 9 on the long arm .4 of distance out from spindle attachment. Linkage order *C-wx*-T with 13.7 percent of crossing over with waxy.

T5-9a (BURNHAM 1934a). Interchange in proximal part of the short arm of chromosome 9. Linkage order *sh-wx*-T with about two percent of crossing over with waxy. Much non-homologous pairing and much suppression of crossing over.

T6-9a (ANDERSON 1934, MCCLINTOCK 1934). Interchange about one-third the way out on the long arm of chromosome 9. Linkage order *C-wx*-T with 11.6 percent of crossing over with waxy.

TRANSLOCATIONS INCLUDED IN THE PRESENT STUDY

In the present paper, data are presented on the following translocations which are included in the writer's published list (ANDERSON, 1935); T 1-9a, b, c, 2-9a, b, 3-9a, b, 4-9a, b, 6-9a, b, and 8-9b. In addition two others are included which will be designated T3-9c and T9-10b. Both were from the writer's X-rayed material (ANDERSON 1935, Lot 1, sixty minute treatment, and Lot 2, fifty minute treatment, respectively). The chromosomes involved were determined by diakinesis observations of intercrosses with known translocations. T3-9c gave two rings of four in crosses with T 1-2a, T 1-7a, T 4-8a, T 5-7a and T 4-5a, and a ring of six with T 8-9a and T 2-3a. T 9-10b gave two rings of four with T 1-7a, T 4-8a, T 4-5a, and T 2-3a, and a ring of six with T 3-9a and T 3-10a. These determinations of chromosomes involved have been checked by cytological observation at mid-prophase and by linkage tests.

No data are presented on T 9-10a as this translocation involves problems connected with the survival of unbalanced gametes and will be reported as a separate paper.

LINKAGE DATA

Since most of the linkage tests with genes from chromosome 9 were made involving the same genes in the same linkage order, the summarized data have been combined in a few tables. The individual cultures have been fairly consistent with the exception of T 4-9a and T 6-9a which are presented in more detail. Except for T 6-9a, backcrosses using the F_1 as male or female have given similar results and are combined in the tables. The arrangement of data in the tables is like that in the linkage summary of EMERSON, BEADLE and FRASER (1935).

Backcross data involving various translocations with the two genes *C* and *wx* are presented in table 1. Data similarly involving the three genes *C*, *sh* and *wx* are presented in table 2. Additional data involving only *wx* are given in table 3 while table 4 gives data involving the genes *C* and *sh* with T 8-9b. In several of the backcross cultures there were large discrepancies between several of the contrary classes, due partly to lower viability of *sh* and *wx*, but chiefly to the presence of yg_2 and one other weak

TABLE 1

Backcross progenies from combinations $\frac{C+T}{+wx+}$.

PARENTAL COMBINATIONS		RECOMBINATIONS						TOTAL	PERCENT RECOMBINATION		COINCIDENCE
		REGION 1		REGION 2		REGIONS 1, 2			1 *c–wx*	2 *wx–T*	
T 1– 9a	154 130	50	45	36	7	1	4	427	23.4	11.2	.44
T 1– 9b	99 68	34	30	76	56	14	15	392	23.7	41.1	.76
T 1– 9c	74 71	24	38	22	1	6	1	237	29.1	12.7	.80
T 2– 9a	42 47	15	19	27	20	9	12	191	28.8	35.6	1.07
T 2– 9b	219 225	54	83	22	20	2	3	628	22.6	7.5	.47
3– 9a	116 55	29	8	5	2	—	—	215	17.2	3.3	—
3– 9b	127 148	45	50	10	11	3	3	397	25.4	6.8	.87
3– 9c	65 65	15	25	14	2	—	1	187	21.9	9.1	.27
4– 9a	76 68	18	43	29	40	9	14	297	28.3	31.0	.88
4– 9a	134 123	46	51	24	16	1	5	400	25.7	11.5	.51
6– 9b	238 220	41	62	15	4	—	3	583	18.2	3.8	.75
8– 9b	31 31	9	11	25	14	4	6	131	22.9	37.4	.89
9–10b	175 126	67	77	14	12	1	—	472	30.7	5.7	.12

chlorophyll gene in some of the *c wx* stocks used. Under somewhat unfavorable weather conditions these proved practically lethal. While theoretically a lethal gene should not alter the linkage percentages, data in-

TABLE 2

Backcross progenies from combinations $\frac{C+\ +T}{+sh\ wx+}$.

PARENTAL COMBINATIONS		RECOMBINATIONS														TOTAL
		REGION 1		REGION 2		REGION 3		1 AND 2		1 AND 3		2 AND 3		1, 2 AND 3		
T 1–9b	132 69	2	7	25	25	66	49			2	2	9	8	1		397
1–9c	145 41	3	3	28	29	24	5	1				1	3			283
2–9a	42 34	3	1	13	15	19	6					3	3	—		139
4–9a	57 21	1	2	17	15	8	2	1				2	3			129
4–9b	68 78	6	1	19	14	1	4	1				1				193

TABLE 3

Backcross progenies from combinations $\frac{+T}{wx+}$.

	PARENTAL COMBINATIONS		RECOMBINATIONS		TOTAL	PERCENT RECOMBINATIONS
2–9a	63	56	31	25	175	32.0
3–9a	202	176	9	6	393	3.8
3–9c	67	66	6	2	141	5.7

TABLE 4

Backcross progeny from $\frac{C+T}{+wx+}$.

	PARENTAL COMBINATIONS		RECOMBINATIONS						TOTAL	PERCENT RECOMBINATION	
			REGION 1		REGION 2		REGIONS 1, 2			*c–sh*	*sh–T*
T 809b	65	55	1	3	28	34	2	1	189	3.7	34.4

volving a lethal gene are not entirely trustworthy for accurate determinations. But for the approximations required in the present work, the data are probably accurate enough.

The T 4-9a progenies fall into two sharply different groups. Three cultures from a single plant, 11-315-2, gave 10.2, 12.2, and 11.6 percent crossing over for the *wx*-T interval. Two cultures from sibs of this plant gave 27.1 and 34.9 percent for the same region. A closely related plant gave 27.6 percent. The two groups of data have been summarized separately. Cytological observations of one plant at mid-prophase of meiosis has shown the interchange of chromosomes about four fifths the way out on the long arm of 9 and very near the spindle attachment on 4. This position would give an expectation of loose linkage corresponding to the higher crossover value. The low value obtained in cultures from 11-315-2 might be due to the presence of some other chromosomal alteration such as the short inversion about the spindle attachment of chromosome 4 which is known to be present in some stocks (McCLINTOCK 1933).

Some further data have been obtained on T 6-9a. Two sister F_1 plants were backcrossed reciprocally with a *c wx* stock. The data are given in table 5. With such small numbers of plants involved, not much reliance can be placed on these data. There is a marked difference in the crossover values when the plants are used as female or male, but the difference is opposite to that previously found for the T -*Y* -*Pl* region of chromosome 6 (ANDERSON 1934). Obviously the crossing-over behavior of T 6-9a needs to be checked more carefully.

TABLE 5

Reciprocal backcrosses of T 6–9a. Parental formula $\frac{C+T}{+wx+}$.

F_1 PARENT	F_1 USED AS	PARENTAL COMBINATIONS		RECOMBINATIONS						TOTALS	PERCENT CROSSING OVER	
				REGION 1		REGION 2		REGIONS 1, 2				
											C–wx	*wx–T*
11–318–1	♀	18	20	8	9	6	7	2	0	70	27.1	21.4
	♂	108	107	26	45	9	2	1	2	300	24.7	4.7
11–318–5	♀	51	52	35	24	4	9	5	3	183	36.6	11.5
	♂	78	17	12	12	3	2	2	1	127	21.3	6.3

CYTOLOGY

Cytological examinations have been made of aceto-carmine preparations at mid-prophase of meiosis. Measurements and estimates of position of the interchange of chromosomes have been made from camera lucida tracings of the clearest figures. The position of the interchange is recorded in tenths of the distance from the spindle attachment to the end of the arm. Thus 9 L.4 indicates chromosome 9 long arm four-tenths of distance from the spindle attachment. In general these placements are probably reliable to about .1 or .2 of the length of the arm. Those near the end of the chromosome are somewhat less reliable. In some cases, notably T 5-9a (BURNHAM 1934a) and T 6-9b, there is much non-homologous pairing (MCCLINTOCK 1933), which makes it difficult to determine the normal position of the interchange.

The three translocations T 8-9a, T6-9a, and T 5-9a have been described. The following have been examined cytologically:

T 1-9b	1 L .6	9 L .5
T 1-9c	1 S .6	9 L .2+
T 2-9a	2 S .7	9 L .6+
T 2-9b	2 S .1	9 L .2
T 3-9a	—	9 L .1+
T 3-9c	3 L .1	9 L .2
T 4-9a	4 L .1	9 L .8
T 4-9b	4 L .6	9 L .2
T 8-9b	8 S .2	9 L .8
T 9-10b	9 L .3	10 near s.a.

T 1-9a and T 3-9b have not been studied. T 6-9b shows much non-homologous pairing, but seems to have the interchange in the proximal part of the long arm of 9.

The writer is indebted to Mrs. GERTRUDE G. FRANDSEN for most of the cytological preparations.

RELATION OF POSITION OF INTERCHANGE TO LINKAGE WITH WAXY

TABLE 6

Comparison of position of interchange with map distance from waxy.

	CYTOLOGICAL POSITION	PERCENT CROSSING OVER FROM *wx*	NUMBER OF PLANTS IN LINKAGE TESTS
T 5-9a	S .1	2.0	(BURNHAM 1934a)
3-9a	L .1+	3.6	608
4-9b	L .2	3.1	193
6-9b		3.8	583
3-9b		6.8	397
2-9b	L .2	7.5	628
3-9c	L .2	7.6	328
1-9c	L .2+	12.1	520
9-10b	L .3	5.7	472
6-9a	L .3	9.4	955
1-9a		11.2	427
8-9a	L .4	13.7	(BURNHAM 1934b)
1-9b	L .5	37.7	789
2-9a	L .6+	30.7	505
4-9a	L .8	31.0	297
		11.5	529
8-9b	L .8	37.4	131

Table 6 gives a list of the interchanges involving chromosome 9 arranged in approximate order of their position in the chromosome and of their crossover distance from waxy. The first column gives the observed position in chromosome 9. The second column gives the percent of crossing over from waxy. The third column gives the total number of backcross plants on which the crossover percentages are based, summarized from the preceding tables. The correlation between the cytological observations and the percentages of crossing over is very close, considering the probable amount of error in the cytological placements. The loose linkages shown by T 1-9b, T2-9a, T 4-9a, and T 8-9b probably represent longer map distances, which can be studied more effectively when one or more helpful genes are found in the distal part of chromosome 9.

SUMMARY

Data are presented on linkage relations with waxy for fourteen translocations involving the long arm of chromosome 9.

The amount of crossing over with waxy is closely correlated with the cytological position of the interchange (table 6).

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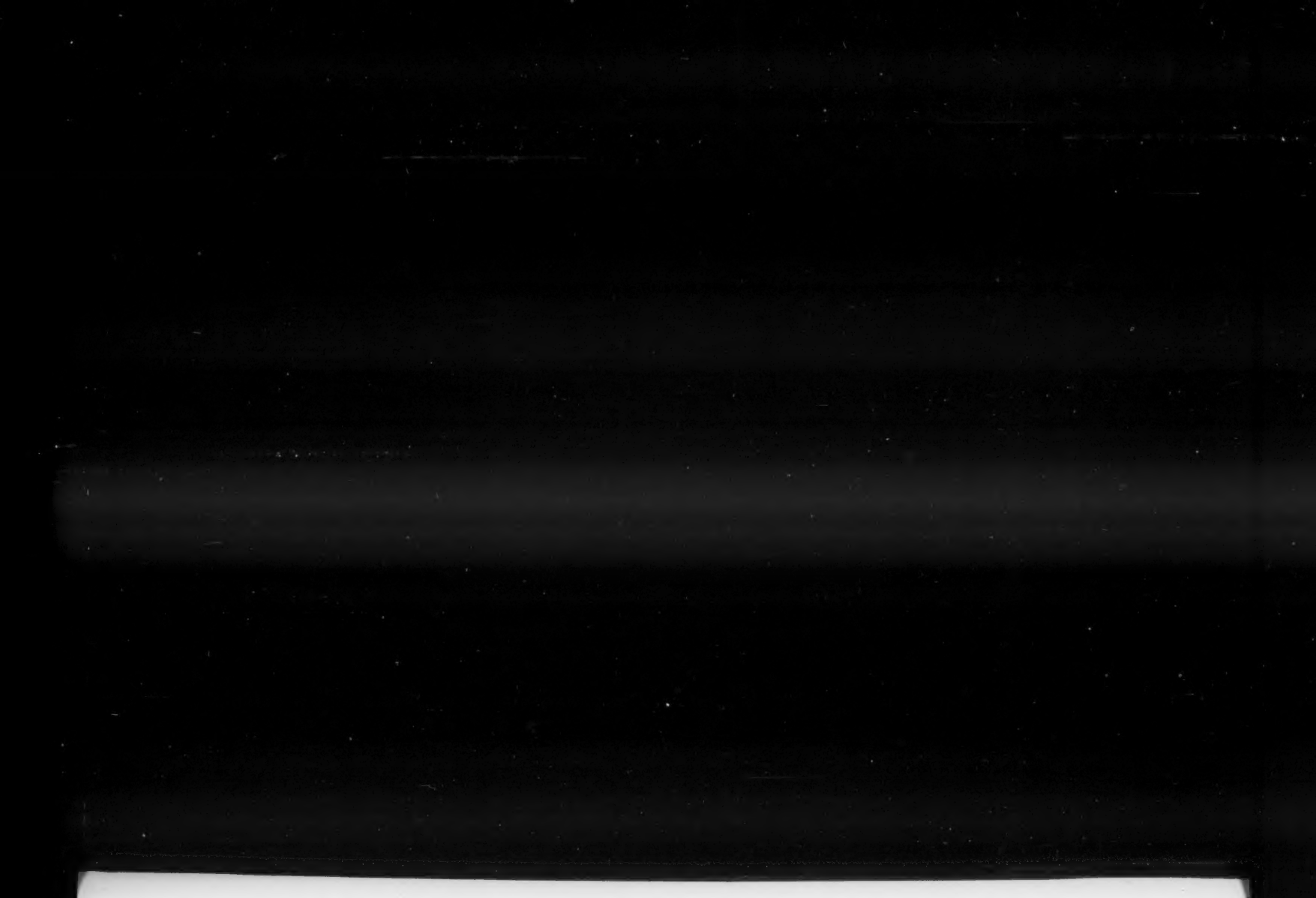
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